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LABORATORY STUDIES WITH STABILIZATION POND ALGAE

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Northwestern University

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LABORATORY STUDIES

WITH

STABILIZATION POND ALGAE

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LABORATORY STUDIES

WITH

STABILIZATION POND ALGAE

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PREFACE

The following is the final report of the investigations carried out as a part of the research project, "Type of Organic Compounds Specifically Affecting Algal Growth." This project was supported by grants RG-5487 and RG-5487 (C1) from the Division of Research Grants, National Institutes of Health, U.S. Public Health Service.

The initial goal of this project was to study the types of organic compounds which affected the growth of unicellular green algae such as are found in stabilization ponds, to investigate the occurance of these compounds in domestic sewage, and to determine the importance of direct algal assimilation of organic compounds in the process of BOD reduction in stabilization ponds. It was found that a variety of organic compounds can affect the growth rate of stabilization pond algae, that some of these compounds do occur in domestic sewage in appreciable quantities, but that the effect of algal metabolism of organic compounds upon stabilization pond operation can not be evaluated until the ecological relationships between the various types of organisms present has been elucidated in great detail.

Investigations of some ecological factors were undertaken in order to evaluate the importance of light intensity, carbon dioxide concentration, pH, and algal concentration upon algal growth and BOD removal in stabilization ponds. Thus this report includes the results of four separate investigations concerning the ecology of stabilization ponds.

Harold B. Gotaas
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THE EFFECT OF ORGANIC COMPOUNDS ON THE GROWTH RATE OF STABILIZATION POND ALGAE

INTRODUCTION

The ecological relationships of any biological waste treatment process are undoubtedly complex. Since a stabilization pond depends upon the presence of autotrophic organisms as well as heterotrophs for proper operation, it is biologically more sophisticated than the other conventional treatment processes.

It has been postulated (1) that in a stabilization the heterotrophic organisms, bacteria, fungi and protozoa, oxidize the organic matter present and excrete carbon dioxide, ammonia, and other inorganic compounds which may serve as nutrients for the autotrophic organisms. The autotrophs, mostly unicellular green algae, in turn produce oxygen, which is utilized by the heterotrophs, as a by-product of their photosynthetic reactions. This hypothesis, although valuable as a starting point for discussions of the ecology of stabilization ponds, is a simplification of the actual situation.

Myers (8) holds that algae can not attain the maximum rate of photosynthesis unless the concentration of carbon dioxide in the gas in contact with the medium is maintained between 1 and 5%. Emerson and Green (9) found that Chlorella could attain a maximum rate of photosynthesis in media in which the concentration of free carbon dioxide ranged from 5×10^{-5} molar to 2×10^{-4} molar. Nielson (10) believes that much of the confusion in regard to the optimum concentration of carbon dioxide is due to the use of cultures in which the algal population density is too great. In very dense cultures the cells do not have equal exposure to the light and the rate of utilization of carbon dioxide is so great that the concentration of carbon dioxide in the culture medium may be a great deal less than the concentration calculated on the basis of equilibrium between the medium and the gases aereating the culture. Using dilute cultures (20,000 cells/ml.) he found that the optimum concentration of carbon dioxide is in the range from 0.1% to 0.03% and that concentrations greater than 1% resulted in the blocking of some enzymatic process.

That some green algae are able to grow heterotrophically in the dark with various sugars serving as the source of carbon and energy has been known for several years. In 1927 Bristol-Roach (2) reported that unicellular green algae were able to utilize glucose, fructose, mannose, galactose, and sucrose as sole carbon sources but he did not determine the growth rate as a function of the carbon source. Aubel (11) found that the growth rate of Chlorella was greater when glucose was the carbon source than in the presence of either fructose or sucrose.

Several investigations have demonstrated that species of Chlorella are able to grow in the dark utilizing various organic acids as the carbon source and may be able to assimilate them in the light. Eny (12) found that lactate, pyruvate, acetate, aconitate, citrate, succinate, fumarate, malate, butyrate, and propionate could all serve as the sole carbon source for Chlorella grown in the dark and that the effect of these compounds on the growth rate is in the order in which they are given above, the growth rate being the greatest on lactate and the least on propionate. However, he also reported that the growth rate of heterotrophically grown Chlorella is much greater on glucose than on any of the acids given above. In later experiments (13) he studied the respiration of Chlorella in the dark by manometric methods and found that the respiratory quotient of the alga was similar to that of many bacteria but the rate of respiration was less. Schlegel (14) demonstrated that the rate of utilization of acetic, pyruvic, malic, and citric acids is greater when the alga is cultured in the light than when it is grown in the dark. Kandler and Ernst (15) reported that many of the acids that are metabolized by algae inhibit growth and respiration when they are present in concentrations greater than 10^{-3} molar.

Watanabe (16) tested the effect of various types of organic compounds on the respiratory rate of Chlorella. He found that various aldehydes and polyalcohols could be respired as well as sugars and organic acids. The greatest respiratory rate was observed in the presence of the sodium salts of fatty acids containing between eight and ten carbon atoms.

Nitrogen Sources

Most of the media that have been used for the cultivation of unicellular green algae contain nitrogen in the form of either ammonium or nitrate ions (4). In a recent review (17) Syrett discussed the assimilation of inorganic nitrogen by green algae. Evidence indicates that green algae will grow equally well employing either nitrate or ammonia as a nitrogen source provided that nitrogen is not the limiting factor. However, nitrogen deficient cells will convert ammonia into amino acid nitrogen much more rapidly than they will assimilate nitrate nitrogen.

It has been shown by several investigators that urea may serve as the sole nitrogen source for algae. Moyse (18) found that Chlorella grew more rapidly on a medium containing urea than on media in which the nitrogen was supplied in the form of either ammonia or nitrate. Champigny (19) showed that urea not only accelerated the growth rate of Chlorella but also increased the percentage of protein in the cells. Ellner and Steers (20) suggested that the more rapid growth of Chlorella on urea might be explained by the fact that the carbon as well as the nitrogen of the compound is utilized by the algae. They were able to demonstrate that the carbon of urea was incorporated into the protoplasm of the cells but that urea could not serve as the sole source of carbon.

Algens (21, 22, 23) studied the utilization of amino acids by several unicellular green algae in a series of growth experiments.

He reported that glycine, alanine, aspartic acid, succinamide, and asparagine could be employed as the sole nitrogen source and that the greatest growth rate was attained using asparagine.

Ludwig (24) found that Chlorella could utilize nitrate, nitrite, ammonia, acetamide, urea, guanidine, uric acid, glycine, alanine, valine, leucine, phenylalanine, glutamic acid, or peptone as the sole nitrogen source. However, hydroxylamine, glucosamine, diphenylamine, p-aminoazobenzene, azobenzene, and diazoaminobenzene were not utilized at all. Arnow et al. (25) reported that ornithine, citruline, arginine, or urea could serve as the sole source of nitrogen for Chlorella vulgaris with no difference in the growth rate as compared to that attained in cultures containing inorganic nitrogen. Asparagine, alanine, cistine, glucine, histidine, hydroxyproline, isoleucine, lysine, methionine, leucine, norleucine, phenylalanine, proline, serine, threonine, tyrosine, valine, and aspartic acid were reported to produce some growth but the cells soon became chlorotic. In addition, it was reported that adenosine triphosphate, glutamic acid, and adenylic acid would markedly stimulate the growth rate.

From the results of the investigations cited above it may be safely concluded that many forms of nitrogen are available to some algae. The disagreement between some of the published reports is probably due to the fact that the cultural conditions were not always the same in different experiments and there is always the possibility that the purity of some of the compounds tested was not the same in each experiment.

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requirements for specific trace elements varies from plant to plant. It has been reported that various algae have requirements for magnesium, manganese, iron, boron, molybdimum, copper, cobalt, and zinc (27). It is possible that other trace elements are required but the concentrations at which they are effective have not been established.

One of the problems of the mass culture of algae has been to devise a method of supplying the trace elements in the proper concentrations and maintaining these concentrations in the medium for a period of several days since many of the ions form insoluble phosphates, carbonates, or hydroxides and they are removed from solution at an appreciable rate by algae. Several authorities (28) have recommended using chelating agents to maintain the proper reserve of trace elements without exceeding the toxic concentration levels.

Waris (29) investigated the effect of adding ethylenediaminetetraacetic acid (EDTA) to cultures of algae and reported that this compound had an accelerating effect on the growth rate over a wide pH range. He ascribed this effect to the chelating properties of the compound as it could maintain the proper concentratios of the trace elements over an extended period of time. However, since EDTA is an alpha amino acid, although not a natural one, there is the possibility that it may be metabolized by the algal cells or by bacteria present as contaminents.

Growth Substances

There are a number of compounds that have the property of accelerating the growth of plants at low concentrations and inhibiting it

at higher concentrations (30). These compounds are referred to as a group as plant growth substances. The growth substance, indoleacetic acid, has been isolated from the tissues of many seed plants and it is believed that it or a closely related compound is required for any plant growth at all to take place. In addition to the naturally occuring growth substances there are a number of synthetic compounds which have growth substance activity.

There has been a great deal of confusion about the effect of the growth substances on the growth rate of algae. Pratt (31) reported that indoleacetic acid, indolebutyruc acid, and indolepropionic acid all stimulated the growth of Chlorella vulgaris and that the greatest acceleration was obtained with indoleacetic acid at a concentration of 50 mg/l. Brannon and Bartsch (32) found that the growth of algae was accelerated by phenylacetic acid, naphthaleneacetic acid, indolebutyric acid, indoleacetic acid, and incolepropionic acid. They reported that the optimum concentration of indoleacetic acid was 10 mg/l. and that it was inhibitory at a concentration of 33.3 mg/l. Leonian and Lilly (33) reported that indoleacetic acid inhibited the growth of Chlorella vulgaria at a concentration of 1 mg/l. and that it was definitely toxic at 10 mg/l. However, the stated purpose of their investigation was to obtain evidence supporting the theory that indoleacetic acid is not a growth promoting substance but an irritant that is actually harmful to plants. This theory has been soundly disproved. In a later series of experiments (34) they were unable to demonstrate an inhibiting effect at concentrations less than 50 mg/l. but also found no definite

acceleration of growth at any concentration. Algeus (35) in an attempt to explain the apparent inconsistencies in the previous experimental work found that indoleacetic acid had no effect on the growth rate of algae when it was present in the ionized form; i.e., in media in which the pH is greater than 7.0. The undissociated acid was found to stimulate cell division if the alga was grown under conditions favorable for the maximum rate of photosynthesis. He found that indoleactic acid did not serve as a source of either carbon or nitrogen. Oxidation products of the acid were found to be toxic and it was reported that indoleacetic acid is rapidly oxidized in an acid solution when exposed to light intensities greater than 100 foot candles.

Yvon (36) studied the effect of naphthaleneacetic acid on the growth of Chlorella pyrenoidosa. She found that it stimulated the growth at a concentration of 10^{-5} molar or 10^{-6} molar when the light intensity was less than 400 foot candles. At higher light intensities the growth was inhibited at these concentrations. At a concentration of 10^{-4} molar the inhibition was independent of the light intensity.

The effect of 2,4-dichlorophenoxyacetic acid on the rate of growth and respiration of Chlorella was investigated by Erickson and his coworkers (37). They reported that it inhibited the respiratory activities at concentrations of 10^{-4} molar when the pH of the medium was 4.0. At higher pH values or when the concentration of 2,4-D was less a stimulation of the respiratory rate was observed. In a later experiment (38) they found that the permeability of the Chlorella cell was 1000 times as great to the acid as to the sodium salt.

Several other compounds that are not normally considered to be growth substances have been found to have an accelerating effect on the growth rate of algae. Ondratschek (39) reported that aneurin, ascorbid acid, and liver extract have a definite growth promoting effect at low concentrations. Van Overbeck (40) found that traumatic acid and thiamine stimulated the growth of algae but that several of the other B-complex vitamines did not. Mai (41) reported that an extract prepared by boiling bananas in water had an accelerating effect on the growth rate of Chlorella which could not be explained by the carbohydrates or any plant acids that were extracted by the boiling water.

Summary

It is quite apparent from the reports of investigations published that unicellular green algae are able to assimilate various types of organic compounds but that the effect of various substrates on the growth rate is still somewhat in doubt. According to Myers (4) the maximum possible growth rate is attained in inorganic media under conditions optimum for photosynthesis and the addition of any organic compound will not accelerate the growth under these conditions. He feels that the reports of an increase in the growth rate in the presence of certain organic compounds is due to the assimilation of carbon which would not occur if the organism were producing carbohydrates at the maximum rate attainable by photosynthesis. However, in view of the reports of acceleration of the growth rate of Chlorella by compounds that do not serve as carbon sources it is quite likely that some organic compounds may stimulate the growth of the algae.

The Organic Materials of Sewage

Since sewage is composed of wastes from all types of human activities it is evident that it will contain a wide variety of organic compounds. The organic compounds and their concentrations in a given sample of sewage will depend upon the community from which the waste water is derived, the season of the year, the time of the day, and the length of time that the sewage has been subjected to bacterial action. It is likely that any organic compound could be found in sewage if the search for it were extensive enough.

The average composition of domestic sewage with a flow of eighty gallons per capita per day may be taken as 480 mg/l. of organic matter of which some 265 mg/l. will be dissolved and the remaining 215 mg/l. will be in form of particulate matter suspended in the flow (42). Naturally the specific values for the concentrations of organic matter in a given sample of sewage may vary somewhat from the above values; however, over a period of time the composition of the sewage from a community will not show so wide a variation that the average values may not be employed in the design of biological treatment processes.

There have been relatively few analyses of the organic components of waste waters, probably because of the labor involved and the uncertainties of analysing complex mixtures of organic compounds. Wedgewood (43) proposed a method of analysis which consisted of extraction of the waste with various organic solvents, chromatographic separation of the compounds in the extracts, and identification of the components by

absorption spectra. He felt that the organic analysis of sewage and industrial wastes would lead to a better understanding of the operation of treatment processes and could indicate methods for the reclaimation of the waste materials in sewage. In an application of the proposed system he was able to demonstrate the occurrence of number of polynuclear hydrocarbons in sewage (44).

Wuhrmann (45) studied the nitrogenous compounds in several samples of sewage and of the effluent from biological treatment processes. He concluded that the greater part of the organic nitrogen was urea and that the free amino acids made up only a small fraction of the total. He found that about 90% of the free amino acids in the raw sewage as determined by the Van Slike method was eliminated by biological treatment.

One of the easiest (and perhaps the poorest) methods of arriving at an estimate of the types of organic compounds present in sewage is to speculate on the materials that are likely to find their way into the sewer. For instance it is obvious that appreciable quantities of soap and synthetic detergents will be present in sewage. If the average consumption of these materials in a community is known it is fairly easy to calculate the concentrations which would be expected to be found in the sewage.

The wates from the kitchen probably make up an appreciable proportion of the organic substances of waste waters. They include a fair amount of grease from animal and vegetable sources including hydrogenated vegetable oils that are used so extensively in cooking. The water

alga to reach its maximum growth rate if the physical conditions are optimum (4). Most of these media contain essentially the same materials but the specific salts employed and the concentrations of the salts vary. The medium selected for this phase of the investigation is a modified Knops solution. The constituents of the medium are given in Table I.

TABLE I

INORGANIC MEDIUM

Nutrient	Supplied as	mg/l. of salt	Molarity x 10^3	mg/l. of nutrient
N	KNO_3	722.0	7.14	100
P	KH_2PO_4	44.0	0.32	10
Mg	$MgSO_4$	75.0	0.62	15
Fe	$Fe2(SO_4)_3$	3.6	0.018	1.0
Ca	$CaCl_2$	1.4	0.012	0.5
B	H_3BO_3	5.4	0.091	1.0
Zn	$ZnSO_4$	1.24	0.0077	0.5
Mn	$MnSO_4$	1.36	0.0091	0.5
Mo	MoO_3	0.75	0.0052	0.5
Cu	$CuSO_4$	1.26	0.0079	0.5
Co	$CoCl_2$-	1.1	0.0085	0.5
S	SO_4-(above)		0.70	22.4
C	CO_2 in air			
	K_2CO_3 and acid	varies		

A series of preliminary experiments was run to determine the optimum concentration of each of the above nutrients and if a small variation in the concentration would effect the growth rate of Chlorella. In the cases of all the nutrients it was found that the concentration of the salt employed could be increased or decreased by a factor of ten without affecting the growth rate.

Cultural Conditions

The conditions under which the organic materials were tested were essentially of three types; namely, 1) culturing in the dark, 2) culturing in the light in the presence of inadequate carbon dioxide concentration, and 3) culturing in the light in the presence of adequate carbon dioxide concentration. By the use of the different cultural conditions it could be determined whether or not an organic material could serve as the sole source of carbon, if it was assimilated in the light, and if it could accelerate the growth rate of the alga under conditions optimum for growth on inorganic media. The source of inorganic nitrogen was omitted from some of the cultures to determine if the organic material being tested could serve as the sole source of nitrogen.

The cultures which were grown in the dark were innoculated directly from slants of Chlorella on sewage agar. The cultures consisted of about 100 ml. of medium placed in a flask with the test material and autoclaved. The cultures were placed in a light-tight cabinet immediately after innoculation. They were inspected for signs of growth at the end of one week and again at the end of the second week. At the end of the third week the cultures were opened and cell counts made on the contents. If Chlorella cells could be detected without microscopic examination at the end of the third week the cultures were taken to be positive. In all cases the distinction between growth and no growth was quite easy to make. Some of the cultures that were taken to be negative showed Chlorella cells on microscopic examination but the cells were present in such small numbers that they could be accounted for by the innoculum.

The cultures which were grown in the light were operated in two different ways. Some of the cultures were made up by placing the culture medium in a flask along with a predetermined amount of acid. The medium was autoclaved and then a solution of potassium carbonate that had been autoclaved separately was added. The reaction of the acid and carbonate released carbon dioxide. The culture was innoculated and allowed to "green up." When the culture was visibly green the test material and more acid to release more carbon dioxide from the unreacted carbonate were added. The day after the addition of the test material a 100 ml. portion of the culture was removed aseptically and the packed cell volume was determined. The next day the packed cell volume was again determined and the growth rate was calculated from the ratio of the two packed cell volume determinations.

The other cultures which were grown in the light were prepared by placing the culture medium in a 500 ml. separatory funnel and autoclaving. The outlet of the funnel was attached to a glass "T" by means of a piece of rubber tubing. One of the ends of the glass "T" was connected with a cotton filter which could be attached to a source of compressed air or 1% carbon dioxide in compressed air.

The other end of the "T" was covered with a piece of rubber tubing that was closed with a pinch clamp. This allowed the culture to be sampled without encountering the danger of contamination. The culture was innoculated and aerated at a slow rate until it turned green. The material to be tested was autoclaved in a small amount of the culture medium and added to the culture after it had turned green. The second day after the addition of the test material an aliquot of the culture was removed and the packed cell volume determined. The packed cell volume was again determined on the following day and the growth rate calculated.

The reason for the two different methods of testing was that although the flask cultures were easier to handle and to maintain free from contamination some authorities are of the opinion that the only way to insure an adequate supply of carbon dioxide is to aerate the cultures with a gas mixture containing 1 - 10% carbon dioxide (4). However, it was found that the growth rate of the Chlorella cells was not dependent upon whether the carbon dioxide was supplied from the air or from the carbonate-acid reaction.

During the testing a number of the cultures were operated without autoclaving or any precaution to assure that they did not become contaminated. The procedure was as described above except that the media was not autoclaved and the cultures were innoculated with a small amount of a concentrated culture that was maintained on the inorganic medium continuously. On the whole very little contamination was observed in the open cultures. However, if the tests indicated that the test material increased the growth rate of Chlorella it was retested in a pure culture to assure that the observed effect was not due to carbon dioxide released by bacteria metabolizing the test material.

The cultures which were grown in the light were supplied light from four, thirty watt, soft white fluorescent bulbs. The light intensity at the surface of the cultures facing the light was about 175 foot candles. The temperature in the cabinet in which the cultures were kept varied between 21 and 28° C during the entire period of experimentation but the variation during any one of the tests was somewhat less than this.

Determination of Packed Cell Volume

Determination of the packed cell volume by centrifuging an aliquot of the culture at a specific force for a specific period of time has been used by a number of investigators as an estimate of the total amount of algal cell material in a culture (4). The use of this determination implies the assumption that the packed cell volume is proportional to the cell population. However, some investigators have held that the

packed cell volume determination is a possible source of error and the only reliable determination of the concentration of algal cell material is the dry weight determination (4). Preliminary experiments indicated that there are several factors other than the algal population that influence the packed cell volume. These factors, other than those that cause relatively large errors but may be easily detected such as the presence of suspended matter other than algae cells and the density of the culture solution, were centrifugal force, time of centrifuging, and the pH of the solution.

The centrifugal force and the time of centrifuging may be easily controlled by the proper operation of the centrifuge. The centrifuge employed in this investigation had a constant speed control and the time of centrifuging was determined by the setting of an electric timer. However, minor variations in these factors were inevitable. The experimental tests were designed so that four cultures were run in parallel and one of the four cultures was a control. The determinations of the packed cell volumes of the cultures in each test were made concurrently so that any variations in the determination could be noted in the results of the control. Any test in which the growth rate of the control varied appreciably from the average growth rate of all the controls was rerun.

A preliminary experiment was undertaken to determine the variation in the packed cell volume of a culture with variation in the pH of the culture at the time of centrifuging. Chlorella cells from several cultures were washed with distilled water, centrifuged, and resuspended in the culture medium. After allowing two days for the cells to reach an

actively growing state in the fresh medium, 100 ml. aliquots of the culture were taken and the pH values of the aliquots were adjusted to the desired values using a concentrated solution of sodium hydroxide (50%) or concentrated hydrochloric acid. The aliquots were placed in Goetz centrifuge tubes and centrifuged at 500 x gravity for twenty minutes. The results of this experiment are shown in Figure 1; the apparent packed cell volume being plotted against the pH of the solution. These data indicate that there is a significant increase in the packed cell volume with an increase in the pH of the culture.

In order to overcome the variation in the packed cell volume with the pH of the culture a procedure was worked out so that the determinations of packed cell volume could be made under standard conditions. The samples from the cultures were centrifuged at 500 x gravity for twenty minutes. The supernatent was decanted and the cells were suspended in 25 ml. of 0.5 molar phosphate buffer at pH 7.0. The buffer suspensions were centrifuged at 500 x gravity for twenty minutes and the packed cell volumes were read from the graduations on the tip of the centrifuge tubes.

From time to time the dry weight of the algae from one of the cultures was determined by washing the cells from the packed cell determination twice in distilled water, drying the cells in a tared crucible at 103° C, and weighing. The data from these determinations are presented in Figure 2, in which the packed cell folume is plotted against the dry weight of the cells. These data indicate that the packed cell volume determinations obtained by the above procedures give a consistent measure of the concentration of algal cell material.

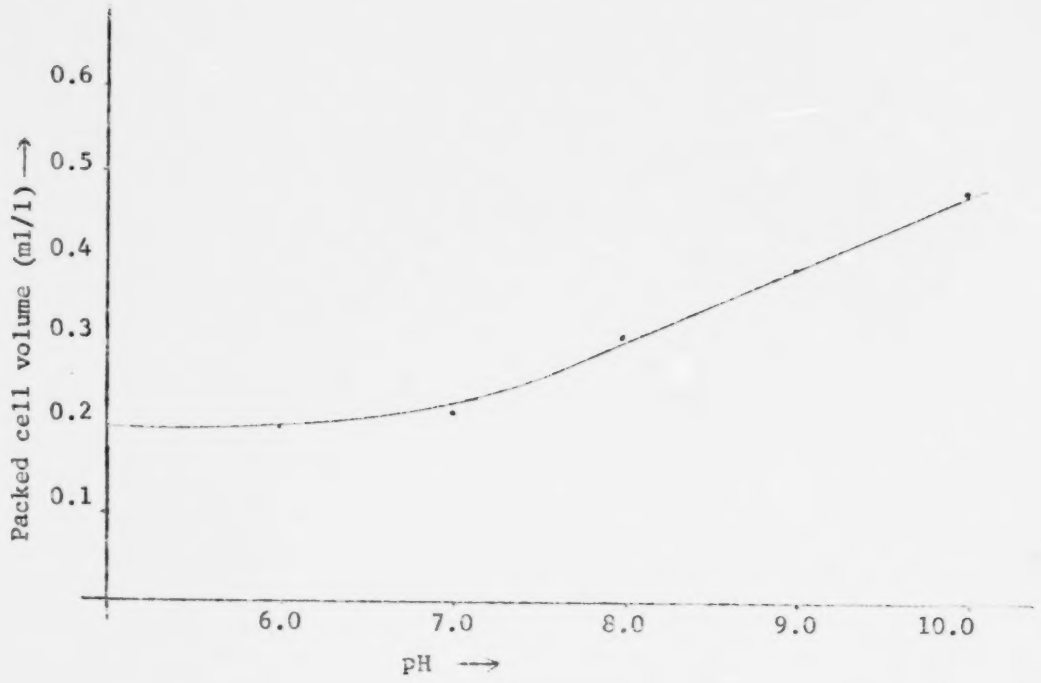

Fig. 1. Apparent packed cell volume as a function of pH.

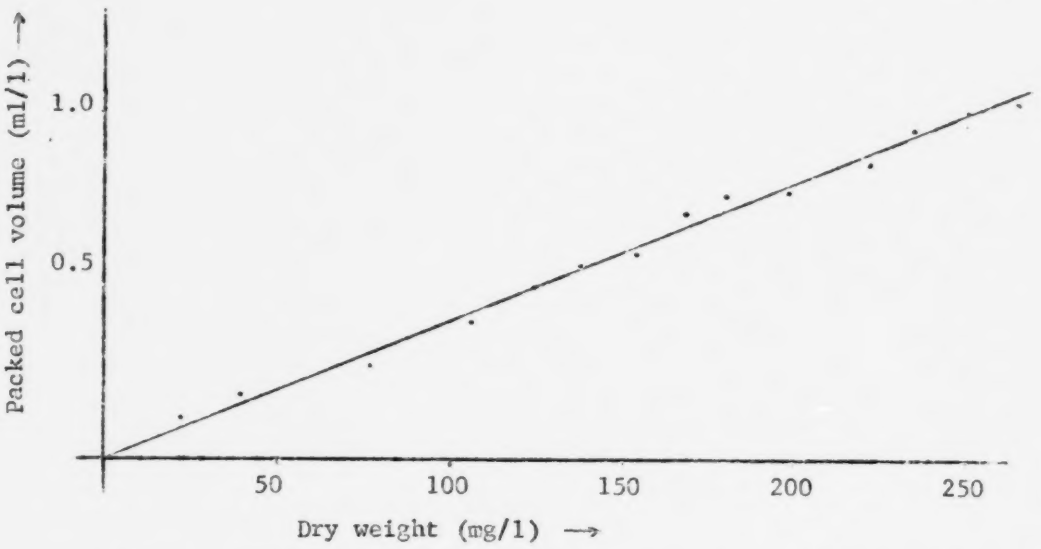

Fig. 2. Correlation between packed cell volume and dry weight.

Fractionation of the Organic Compounds of Sewage

Several samples of sewage were collected from the North Side Sewage Treatment Plant of the Metropolitan Sanitary District of Chicago to be used in the determination of the types of organic compounds occurring in sewage that have an effect on the growth of Chlorella. A total of six samples, each sample having a volume of about five gallons, were collected on weekday mornings. The sewage was derived from a district that is primarily residential but also includes some light industry and several commercial establishments. About one liter out of each sample was sterilized by autoclaving so that it could be used later to determine the effect of the sewage itself on the growth rate of Chlorella. The remainder of each sample was preserved by adding 500 ml. of ethyl ether and storing in a refrigerator until the extraction could be completed. The extraction of each sample took about two days to complete.

The extraction procedure is diagrammed as a flow sheet in Figure 3. The extraction separates the organic components of sewage into six fractions on the basis of the solubilities in water, ether, acid, or base. The compounds that are more soluble in water at a neutral pH than in ether will remain in the sewage when it is extracted with ether. These compounds were labeled as Fraction W as indicated in Figure 3.

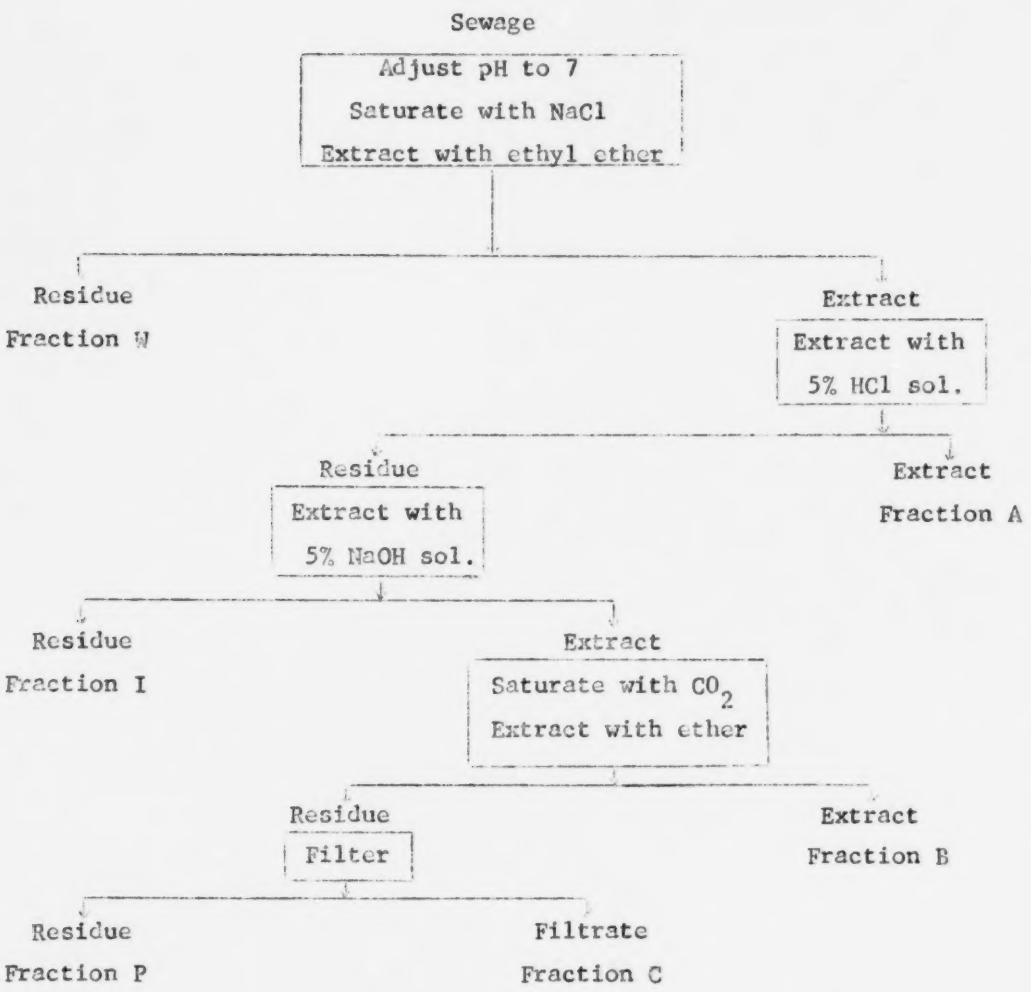

Fig. 3. Flow diagram of the extraction process.

chlorophyll. No difference in the growth rate in those cultures in which nitrogen was supplied in the form of ammonia from that of those in which nitrate was present was observed.

The concentration of carbon dioxide supplied to the algae was found to have an effect on the growth rate. The concentration of free carbon dioxide in the cultures which were aerated was calculated on the assumption of equilibrium between the carbon dioxide dissolved in the medium and the partial pressure of the carbon dioxide in the gas used to aerate the culture using Henry's law. The concentration of carbon dioxide in the other cultures at the beginning of the growth period may be calculated from the amount of potassium carbonate added (1 gm/l.) and the pH of the culture after the addition of the acid. The conditions for equilibrium in a solution containing carbonate, bicarbonate, and free carbon dioxide are that the equations

$$\frac{[H^+][HCO_3^-]}{CO_2} = K_1 = 4.45 \times 10^{-7}$$

$$\frac{[H^+][CO_3^=]}{[HCO_3^-]} = K_2 = 4.69 \times 10^{-11}$$

and $$[H^+][OH^-] = K_w = 10^{-14}$$

are satisfied simultaneously. These equations may be solved to give the percentages of the total present in the form of carbon dioxide, bicarbonate, and carbonate as a function of pH as shown in figure 4. Using this plot it may be calculated that if 1 gm/l. (0.00725 M.) of K_2CO_3 is added

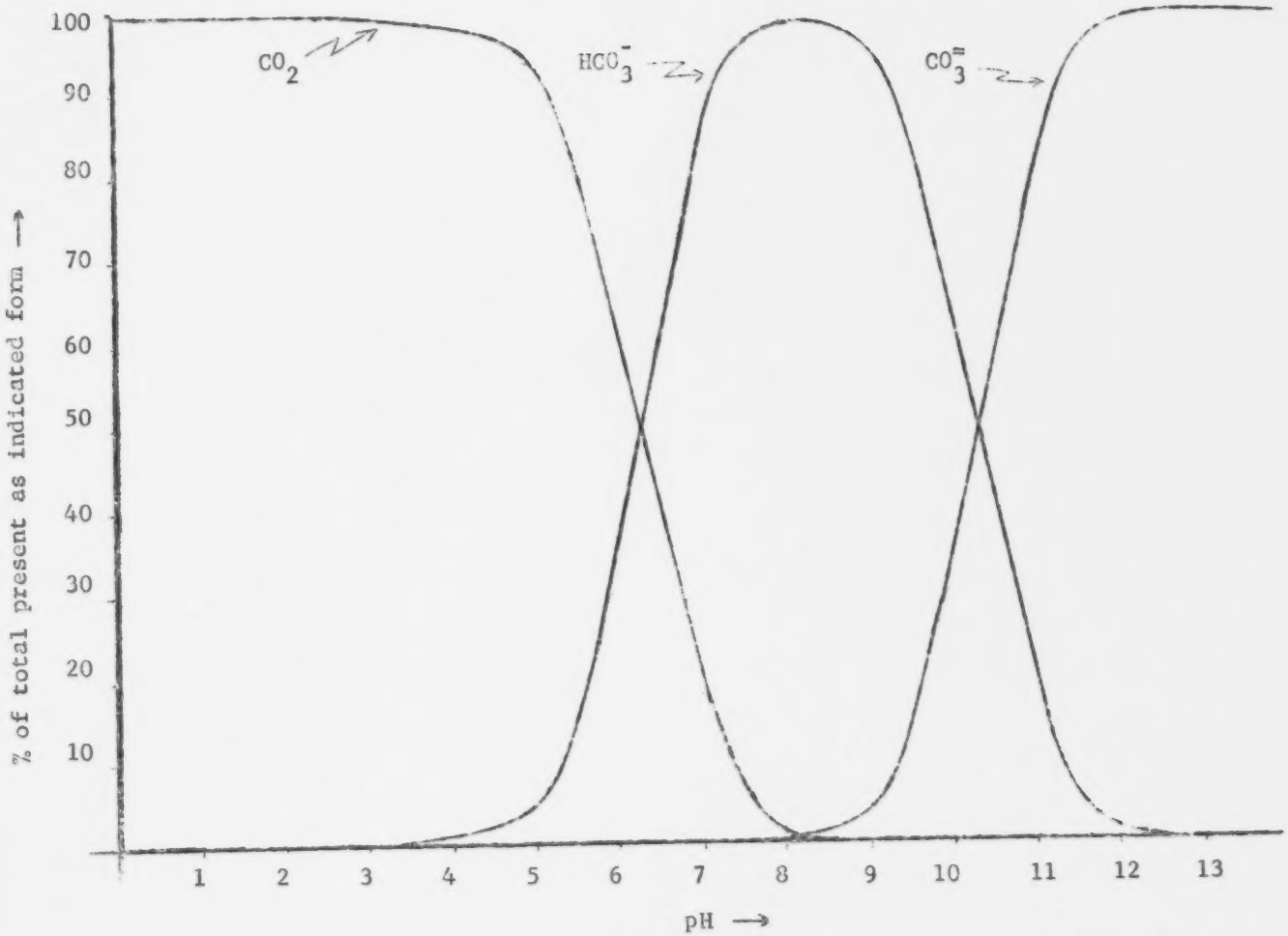

Fig. 4. Carbonate-bicarbonate-carbondioxide buffer system as a function of pH.

to a solution and the pH of the solution adjusted to 6.9 the solution will contain 0.11 x 0.00725 = 0.000795 moles per liter or 35 mg/l. of free carbon dioxide. As the carbon dioxide is used up by the algae more bicarbonate will ionize but at the same time the pH of the solution will increase so that the overall effect is to decrease the concentration of carbon dioxide. The average concentration of carbon dioxide in a culture starting at pH 6.9 and 35 mg/l. carbon dioxide over the time until the pH reaches 8.5 is about 18 mg/l.

Another preliminary experiment was undertaken to determine the effect of the population density at the beginning of the growth period on the growth rate of the Chlorella. The cells used for the innoculation of the cultures were pregrown, harvested by centrifuging, and resuspended in the culture medium to give the desired concentrations. Again no attempt was made to prevent contamination. The results from these cultures are presented in Table III. These results indicate that in the range between 0.08 and 0.42 ml/l. packed cell volume of Chlorella cells in the culture the growth constant does not vary as long as no nutrient becomes limiting. The lower rate of growth at higher cell concentrations is probably due to mutual shading of the cells.

TABLE III

EFFECT OF CHLORELLA CELL CONCENTRATION ON GROWTH RATE

Initial Packed Cell Volume (ml/l.)	Final Packed Cell Volume (ml/l.)	Growth Rate (K_g)
0.03	0.15	1.610
0.08	0.32	1.385
0.18	0.70	1.360
0.30	1.22	1.405
0.42	1.68	1.385
0.54	2.00	1.310
0.72	2.20	1.118

Cultures with Inadequate Carbon Dioxide

The first series of tests of the effect of organic compounds on the growth rate of Chlorella pyrenoidosa was carried out in open cultures in which the concentration of carbon dioxide was 1.5 mg/l. at the beginning of the growth period. The results are compiled in Table IV. These results indicate that the growth rate of the algae was accelerated in the presence of many of the compounds tested. However, since bacteria were present and the supply of carbon dioxide was inadequate the interpretation of any of the test results to indicate that a given compound was assimilated directly by the Chlorella cells is unwarranted. These experiments were carried out mainly to indicate which compounds had no effect at all on the growth rate of the alga and could be eliminated from consideration.

The aromatic acids tested apparently did not have any effect on the growth rate at all. The fatty acids had little effect on the growth rate of Chlorella except for formic and acetic acids which apparently inhibited the growth rate at concentrations of 10^{-3} molar and stimulated it at a lesser concentration. The dicarboxylic acids showed evident stimulation at all concentrations tried as did pyruvic, lactic, and citric acids. It has been known for some time that all of these acids are important in the metabolism of higher plants and it would not be surprising if they were assimilated by Chlorella.

Of the nitrogen containing compounds tested all except the simple aliphatic amines gave good acceleration of the growth rate. The stimulation in this case could be due to the release of carbon dioxide by

TABLE IV

TESTS OF PURE ORGANIC COMPOUNDS IN OPEN CULTURES WITH INADEQUATE CARBON DIOXIDE SUPPLY

Compound	Concentration			
	0	10^{-3}M.	10^{-4}M.	10^{-5}M.
	Growth Rate			
Acids				
Formic	0.364	0.329	0.530	0.405
Acetic	0.371	0.307	0.488	0.418
Propionic	0.355	0.323	0.388	0.374
Butyric	0.348	0.344	0.364	0.360
Valeric	0.358	0.371	0.356	0.371
Oxalic	0.374	0.832	0.666	0.483
Malonic	0.364	0.882	0.703	0.452
Tartaric	0.358	0.895	0.695	0.371
Succinic	0.366	0.878	0.717	0.318
Pyruvic	0.374	0.866	0.644	0.486
Lactic	0.348	0.846	0.722	0.456
Citric	0.364	0.920	0.886	0.522
Phthalic	0.344	0.348	0.348	0.352
Benzioc	0.372	0.322	0.354	0.348
Salicylic	0.354	0.362	0.371	0.362
Acetylsalicylic	0.364	0.364	0.358	0.371
Alcohols				
Methyl	0.372	0.366	0.364	0.358
Ethyl	0.358	0.374	0.358	0.358
Propyl	0.364	0.348	0.355	0.372
Amines				
Methyl	0.352	0.358	0.348	0.352
Ethyl	0.354	0.352	0.364	0.364
Propyl	0.366	0.364	0.358	0.352
Amino Acids				
Glycine	0.364	0.895	0.642	0.406
Alinine	0.354	0.824	0.789	0.424
Glumatic acid	0.348	0.904	0.858	0.488
Tryptophane	0.370	0.894	0.882	0.412
Tyrosine	0.360	0.856	0.802	0.464
Phenylalanine	0.366	0.872	0.864	0.426
Growth Substances				
Indoleacetic acid	0.364	0.388	0.392	0.378
Indolebutyric acid	0.348	0.344	0.372	0.388
Naphthaleneacetic acid	0.354	0.356	0.358	0.412
2,4-dichlorophenoxy-acetic acid	0.370	0.342	0.368	0.364
Miscellaneous				
Hydrolysed Casine	0.344	0.788	0.706	0.398
Yeast Extract	0.368	0.622	0.586	0.378
Ascorbic Acid	0.358	0.882	0.788	0.468
Urea	0.368	0.922	0.865	0.498
Uric acid	0.352	0.786	0.712	0.424

bacterial activity but since the greatest acceleration of the growth rate was observed with urea, which is not a good substrate for bacterial growth, it is possible that some of the organic nitrogen was being assimilated directly by the Chlorella. The stimulation observed with yeast extract may be due to the presence of an organic nitrogen source rather than to the effect of any of the vitamins present.

The growth substances tested did not show any acceleration of the growth rate under these conditions. On the other hand, no inhibition was observed either. It is likely that growth substances would not have any effect on the growth rate of algae unless conditions were optimum for growth to begin with.

Table V lists the results of a series of tests carried out in pure cultures in which the concentration of carbon dioxide was less than that at which the maximum growth rate is attacined. Stimulation of the growth rate under these conditions could not be due to the assimilation of extra carbon dioxide since no bacteria were present. In most instances it should indicate direct utilization of the compound in question as a carbon and/or nitrogen source.

The results of these tests almost paralleled those of the first series indicating that Chlorella pyrenoidosa is able to metabolize a variety of organic compounds as additional carbon sources when the supply of carbon dioxide is inadequate. Again the greatest stimulation of the growth rate was observed in the presence of organic nitrogen sources, especially urea.

TABLE V

TESTS OF ORGANIC COMPOUNDS IN PURE CULTURES WITH INADEQUATE CARBON DIOXIDE SUPPLY

Compound	Concentration 0	10^{-3}M.	10^{-4}M.	10^{-5}M.
	Growth Rate			
Acids				
Formic	0.348	0.332	0.508	0.418
Acetic	0.364	0.328	0.492	0.392
Oxalic	0.354	0.856	0.644	0.490
Malonic	0.370	0.872	0.718	0.444
Tartaric	0.366	0.880	0.708	0.405
Succinic	0.348	0.862	0.734	0.370
Pyruvic	0.356	0.882	0.688	0.462
Lactic	0.344	0.868	0.708	0.424
Citric	0.364	0.892	0.876	0.514
Amino Acids				
Glycine	0.344	0.942	0.888	0.422
Alanine	0.366	0.878	0.818	0.388
Glutamic acid	0.377	0.898	0.900	0.502
Tryptophane	0.348	0.846	0.814	0.464
Phenylalanine	0.360	0.875	0.718	0.443
Growth Substances				
Indoleacetic acid	0.340	0.382	0.388	0.396
Indolebutyric acid	0.366	0.360	0.368	0.472
Naphthaleneacetic acid	0.378	0.384	0.388	0.372
2,4-dichlorophenoxy-acetic acid	0.345	0.410	0.392	0.383
Miscellaneous				
Hydrolysed Casine	0.375	0.734	0.638	0.418
Yeast Extract	0.354	0.600	0.578	0.348
Ascorbic acid	0.368	0.922	0.824	0.446
Urea	0.368	0.902	0.898	0.512
Uric acid	0.374	0.454	0.432	0.378

Cultures with Adequate Carbon Dioxide

The compounds which were found to accelerate the growth rate of Chlorella pyrenoidosa in pure cultures in which the concentration of carbon dioxide was inadequate were tested in the presence of adequate carbon dioxide. The results of these tests are presented in Table VI.

TABLE VI

TESTS OF ORGANIC COMPOUNDS IN PURE CULTURES WITH ADEQUATE CARBON DIOXIDE SUPPLY

Compound	Concentration 0	10^{-3}M.	10^{-4}M.	10^{-5}M.
	Growth Rate			
Acids				
Formic	1.390	1.015	1.344	1.385
Acetic	1.410	1.180	1.380	1.390
Oxalic	1.460	1.410	1.385	1.395
Malonic	1.345	1.380	1.390	1.360
Tartaric	1.370	1.355	1.340	1.385
Succinic	1.400	1.385	1.385	1.385
Pyruvic	1.390	1.355	1.380	1.380
Lactic	1.360	1.360	1.380	1.355
Citric	1.385	1.370	1.370	1.390
Amino Acids				
Glycine	1.440	1.670	1.655	1.410
Alanine	1.385	1.595	1.610	1.510
Glutamic acid	1.390	1.700	1.670	1.455
Tryptophane	1.360	1.655	1.670	1.420
Tyrosine	1.385	1.720	1.690	1.385
Growth Substances				
Indoleacetic acid	1.355	2.080	2.015	1.460
Indolebutyric acid	1.390	1.945	1.970	1.480
Naphthaleneacetic acid	1.410	1.900	1.945	1.410
2,4-dichlorophenoxy-acetic acid	1.420	1.860	1.860	1.390
Miscellaneous				
Hydrolysed Casine	1.410	1.690	1.620	1.410
Yeast Extract	1.340	1.460	1.455	1.410
Ascorbic acid	1.385	1.390	1.380	1.410
Urea	1.385	1.860	1.860	1.390
Uric acid	1.370	1.440	1.410	1.390

It may be seen that none of the organic acids were found to accelerate the growth rate when carbon dioxide was not limiting. However, many of the compounds that could serve as nitrogen sources or as sources of both carbon and nitrogen accelerated the growth rate although the percentage

increase was not as great as in the cultures in which the carbon dioxide concentration was inadequate. All of the growth substances tested were found to accelerate the growth rate of the alga in the presence of adequate carbon dioxide although no definite stimulation of growth had been observed previously.

Testing of Sewage Organics

Cultures Grown in the Dark

The results of the cultures which were grown in the dark are reported in Table VII. The cultures which had population densities less than 20,000 cells per ml. were taken to indicate no growth since the cultures were innoculated with this dense a population. From the results of the cultures in which Chlorella grew it may be seen that citrate could support the growth of the alga in the presence of the required inorganic elements.

TABLE VII

TESTS OF GROWTH IN THE DARK

	Population at 3 weeks (cells/ml. x 10^{-6})
Inorganic plus citrate (0.001 M.)	6.28
Inorganic plus urea (0.001 M.)	----
Inorganic plus indoleacetic acid (0.001 M.)	----
Inorganic plus citrate (0.001 M.) plus urea (0.001 M.)	10.17
Inorganic plus citrate (0.001 M.) plus urea (0.001 M.) plus indoleacetic acid (0.001 M.)	12.87
Sewage	9.27
Fraction W	3.32
Inorganic plus Fraction A	----
Inorganic plus Fraction I	----
Inorganic plus Fraction B	0.72
Inorganic plus Fraction P	----
Inorganic plus Fraction C	1.87

Neither urea nor indoleacetic acid could support growth alone but both increased the rate of growth when citrate was present to serve as the carbon source. The materials isolated from sewage were diluted so that the compounds present were in approximately the same concentrations that they were present in the original sewage. Sewage alone could support growth indicating that it contains compounds that may serve as carbon sources for the algae. Extraction of the sewage with ethyl ether apparently did not remove all of the compounds that serve as carbon sources but did decrease the total amount of substrate. Of the other fractions only those containing compounds which are soluble in base were able to support growth indicating that many of the organic compounds in sewage which are available as carbon sources for Chlorella are organic acids.

Cultures Grown in the Light

The results of the experiments in which the effect of the various fractions from sewage on the growth rate of Chlorella pyrenoidosa was determined are presented in Tables VIII and IX. For these tests the

TABLE VIII

TESTS OF SEWAGE ORGANICS IN PURE CULTURES WITH INADEQUATE CARBON DIOXIDE

Material	Dilution			
	Control	1:0	1:10	1:100
	Growth Rate			
Sewage	0.364	1.065	0.722	0.384
Urine(diluted 1:10)	0.348	1.100	1.030	0.866
Fraction W	0.372	0.889	0.646	0.397
Fraction A	0.375	0.368	0.354	0.368
Fraction I	0.368	0.298	0.348	0.372
Fraction B	0.362	0.464	0.368	0.374
Fraction P	0.365	0.386	0.375	0.354
Fraction C	0.352	0.512	0.408	0.366

concentrations they inhibit algal growth.

It has been shown that sewage contains material which will increase the growth rate of algae over that attained in cultures on inorganic media if the rate of photosynthesis is limited by low carbon dioxide concentration. Most of this material is not extracted from sewage with ethyl ether. The portion which is extracted is acidic in nature. Thus, it would seem that the organic compounds of sewage which are metabolized by algae serve as carbon sources or as nitrogen sources. There is some evidence that the plant growth hormone, indoleacetic acid, occurs in sewage but apparently at concentrations which are so small that it has no effect on algal growth.

It has also been shown that fresh urine diluted 1:1000 is a better source of organic substrates than sewage after it has reached the treatment plant. Apparently the organic compounds of sewage which may be metabolized by algae are also broken down by bacteria and stale sewage or sewage subjected to biological treatment would contain less substrate for algae than would fresh sewage.

It is not likely that the conditions in a stabilization pond would be optimum for photosynthesis for more than a very small fraction of the time. At night photosynthesis stops because of the lack of light. The common observation that the pH of a stabilization pond increases rapidly during the daytime is good evidence that the carbon dioxide concentration in the pond is usually much smaller than that required for maximum photosynthetic rates.

When the algae in a stabilization pond have their rates of growth and photosynthesis limited by either light intensity or by carbon dioxide concentration they will be able to use some of the organic matter present as an additional carbon source. Under all conditions they will be able to use some of the organic nitrogen compounds. However, there are undoubtedly a large number of organic compounds in sewage which algae are unable to metabolize and the algae have to compete with bacteria, which have much greater metabolic rates, for the compounds which they can use. Thus it would be very difficult to predict the role which the organic nutrition of the algae plays in the stabilization of waste and the growth of algae in a stabilization pond unless some method of separating the effect of the algae and the effect of the bacteria on the environment is devised.

CONCLUSIONS

1. Several types of organic compounds such as acids, amino acids, and some nitrogenous compounds will accelerate the growth rate of Chlorella pyrenoidosa when the rate of photosynthesis is limited by the concentration of carbon dioxide indicating that these compounds may be used as carbon and/or nitrogen sources.

2. Organic nitrogen sources and growth substances will accelerate the growth rate of Chlorella pyrenoidosa above that attained on inorganic media even when the conditions for photosynthesis are optimum.

3. The organic compounds which are commonly found dissolved in sewage will accelerate the growth rate of Chlorella above that attained in inorganic media when carbon dioxide concentration is limiting indicating that some of these compounds may serve as carbon sources.

4. The organic compounds of sewage which may serve as carbon sources for Chlorella pyrenoidosa are either more soluble in water having a neutral reaction than in ethyl ether indicating that they are polyfunctional compounds such as carbohydrates, hydroxyacids, ketoacids, amino acids, or urea or they form salts in alkaline solution indicating that they are carboxylic acids.

5. Some of the organic compounds of sewage will accelerate the growth rate of Chlorella pyrenoidosa over that attained on inorganic media under conditions optimum for photosynthesis.

GROWTH OF ALGAE IN CONTINUOUS CULTURE

INTRODUCTION

From the results reported in Section I of this report it is evident that many organic compounds which may serve as carbon sources for algae are present in sewage. However, the algae in a stabilization pond will not utilize these materials if the conditions in the pond are optimum for photosynthesis. If the growth rate of the algae is limited by either carbon dioxide concentration or by light intensity the organic material may be used as an additional carbon source by the algae. Thus it will be necessary to be able to predict the fraction of the time during which algal growth is either light limited or CO_2 limited in order to determine the importance of the organic nutrition of the algae in the ecology of a stabilization pond.

Accordingly, an investigation was undertaken to develop an analytical model which would describe the conditions of growth in a stabilization pond. Ideally, the model should describe the growth and metabolism of the algae and of the heterotrophic organisms, bacteria, fungi, and protozoa, in terms of such environmental perameters as rate of sewage flow, sewage strength, temperature, light intensity, area and

depth of the pond, etc. From the model it should be possible to calculate the population density of the algae and the heterotrophs in the pond, the rate of BOD removal, and the concentration of BOD in the effluent as functions of the perameters describing the environment.

Oswald and Gotaas (1) developed a model to describe waste treatment in stabilization ponds on the assumption that the total amount of visible light energy absorbed by the pond was the controlling factor. They related algal growth and oxygen production to available light energy and derived an equation relating the optimum depth - detention period ratio to the available light energy, the photosynthetic efficiency of the algae, and the BOD of the influent sewage. This model appears to describe the overall operation of certain types of stabilization ponds very well. However, this model does not describe the complex ecological interactions of a stabilization pond and it is expected that a knowledge of these relationships could result in a more rational approach to stabilization pond design and operation.

Hermann and Gloyna (47) developed a model describing the removal of BOD in a stabilization pond as a function of the temperature and loading of the pond. They assumed that light intensity and algal population in the pond would have no effect upon the treatment efficiency attained. If their model is valid it should be possible to show by a detailed consideration of the ecological relationships between the algae and heterotrophs in a stabilization pond that the presence of algae in the pond has no effect upon the growth and metabolism of heterotrophs.

An improvement on the presently available models of a stabilization pond would have to be rather sophisticated in its treatment of the ecological relationships. The approach taken herein was to start with the simple case of algal growth in pure cultures on inorganic media. Once a satisfactory description of this simple case is available it should be possible to develop treatments of the situations which occur when algal cultures or mixed cultures of algae and heterotrophic organisms are grown on organic media.

THEORY

Bench scale continuous cultures have been used as models of mass algal cultures and for studies of algal growth and physiology. According to Krauss (48) the most useful laboratory device for the continuous culture of algae is the Chemostat (49), which is essentially identical with the Bactogen (50). In order to interpret the results obtained with this device, it is necessary to have a mathematical model which describes the growth rate of the organism being cultured as a function of the environmental factor or factors which are limiting.

The Chemostat consists essentially of a culture vessel to which nutrient medium is fed at a constant rate. An over-flow device maintains the rate of effluent withdrawal equal to the rate of feed and thus the culture is held at a constant volume. The culture is mixed continuously so that influent medium is dispersed uniformly throughout the culture and so that the instantaneous population density of the effluent is identical with that of the culture. Accessory equipment

includes constant temperature control devices, some means of aeration and, when used for algal growth, a source of light. A schematic diagram of a Chemostat is shown in figure 5.

The theory of growth in a Chemostat was developed by Novick and Szilard (49) and by Monod (50). The original mathematical model has been verified both mathematically and experimentally, and has been elaborated to cover a number of special applications. Novick (51) reviewed the theoretical developments and Gerhardt and Bartlett (52) reviewed the industrial applications of continuous cultures of bacteria.

TABLE X

SYMBOLS USED IN MATHEMATICAL ANALYSIS

Description	Symbol	Derivation	Units
Volume of Culture	V		liters
Time	t		days
Feed Rate	w		liters per day
Retention Time	t_r	V/w	days
Population Density	N		per liter
Concentration of Substrate			
In Feed	a		gm per liter
In Culture	c		gm per liter
Specific Growth Rate	k	(1/N)(dN/dt)	per day
Saturation Growth Rate Constant	G	max. value of k	per day
Growth yield	q		per gm
Production Rate	P	N/t_r	per liter per day
Rate of Supply of Limiting Nutrilite	r		gm per day
Light Limited Growth Rate	K	(dN/dt)	per day

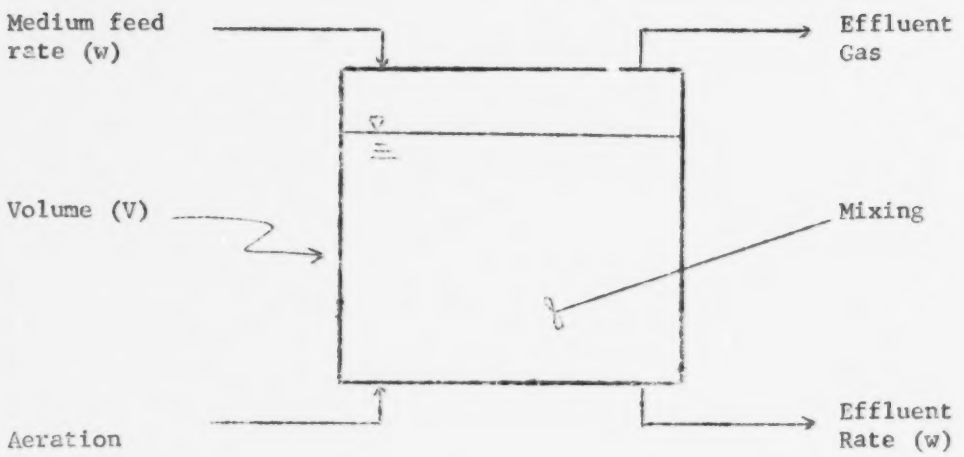

Fig. 5. Chemostat (schematic).

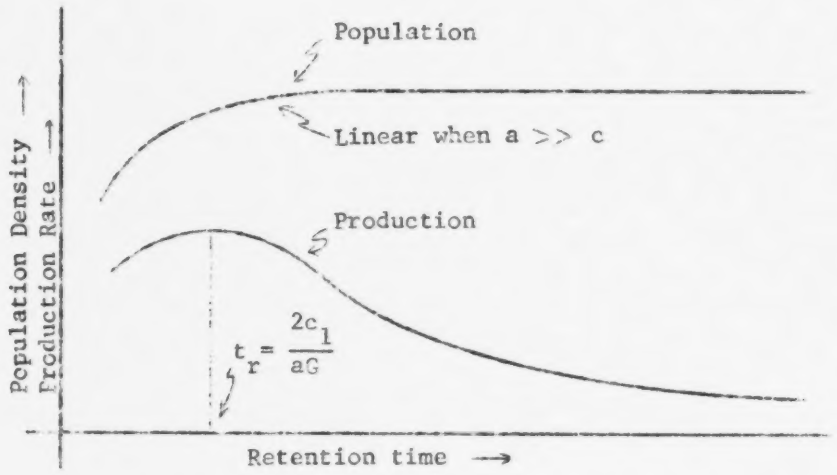

Fig. 6. Expected variation of population density and production rate in a Chemostat.

As noted by Gerhardt and Bartlett (52), the symbols and terminology employed to describe continuous microbial cultures is somewhat confusing because various authors have developed their own systems. The symbols employed herein are given in Table X and have been adopted from those of Novick (51), with a few additions for terms which he did not employ.

An essential feature of the Chemostat is that the medium fed is so designed that all nutrients except one are present in excess. In general, the growth rate of the organism will be a function of the concentration of the limiting nutrient in the culture. Monod (53) found that it was reasonable to express this function as:

$$k = G \frac{c}{c_1 + c}$$

where k is the specific growth rate at concentration c, G is the saturation growth rate constant, and c_1 is the concentration at $k = 1/2\ G$. If c is much, much less than c_1, as normally occurs in a Chemostat, then this expression reduces to

$$k \cong G \frac{c}{c_1} \qquad 1.$$

Monod (53) stated also that in many cases the number of cells produced in a batch culture is directly proportional to the amount of limiting nutrient utilized or

$$N - N_o = q\ (a - c)$$

where N and N_o are the final and initial concentrations of organisms, respectively, q is the growth yield constant (number of cells produced

per gram of nutrient utilized) and a and c are the initial and final concentrations of the limiting nutrient respectively.

The growth of a microbial culture which is limited by the concentration of a single nutrient is described by

$$\frac{dN}{dt} = kN$$

However, in a Chemostat the organisms are being removed at a rate given by

$$N \frac{v}{V} = \frac{N}{t_r}$$

Thus the differential equation for the change in population density in a Chemostat is

$$\frac{dN}{dt} = kN - N/t_r = (k - 1/t_r)\, N \qquad 2.$$

The differential equation for the change in concentration of the limiting nutrient in a Chemostat is

$$\frac{dc}{dt} = \frac{a}{t_r} - \frac{c}{t_r} - \frac{kN}{q} \qquad 3.$$

When a Chemostat culture reaches equilibrium both $\frac{dN}{dt}$ and $\frac{dc}{dt}$ are equal to zero and $k = 1/t_r$.

Spicer (54) proved mathematically that the system of equations 1, 2, and 3 was self-stabilizing and free from oscillations due to overshooting. Moser (55) solved the equations simultaneously and found that at equilibrium

$$N = q(a - c) \cong qa \qquad 4.$$

and

$$c = c_1/Ct_r$$

Novick and Szilard (49) and Monod (50) had previously verified Moser's conclusions experimentally.

If the goal of a Chemostat culture is the production of the maximum number of organisms, then there would be an interest in rate of production. Production rate may be defined as the equilibrium population density divided by the retention time

$$P = \frac{N}{t_r} = \frac{a(a-c)}{t_r} \cong \frac{qa}{t_r} \qquad 6.$$

The maximum rate of production will occur

when $$\frac{dP}{dt_r} = -\frac{qa}{2} + \frac{2q-c_1}{Ct_r^3} = 0$$

or when $$t_r = \frac{2c_1}{aC}$$

At longer retention times, c will become small enough that it may be neglected in comparison with a and then the equilibrium population density may be approximated by qa and the production rate by qa/t_r. These conclusions are shown graphically in figure 6.

In continuous cultures of algae on an inorganic medium, the provision of adequate concentrations of all nutrients in the feed medium is relatively simple compared with the problems of providing adequate carbon dioxide and optimum light intensity. Much of the experimental work on continuous algal cultures has centered on these two problems (56). It is proposed to extend the theory developed to describe the growth of microorganisms in a Chemostat to continuous cultures in which a nutrilite, introduced independently of a medium fed, is limiting and to a continuous culture in which light intensity is limiting.

continuous culture is described by

$$\frac{dN}{dt} = \frac{K}{V} - \frac{N}{t_r} \qquad 10.$$

This is a linear differential equation which may be integrated to give:

$$Ne^{t/t_r} = \frac{Kt_r}{V} e^{t/t_r} + C$$

The constant of integration may be evaluated by taking the boundary conditions $t = 0$ when $N = N_s$, the population density at which light becomes limiting.

Then $$C = N_s - \frac{K t_r}{V}$$

and $$N = \frac{K t_r}{V} + \left(N_s - \frac{K t_r}{V}\right) e^{-t/t_r} \qquad 11.$$

The effect of the term $(N_s - K t_r/v)e^{-t/t_r}$ may be evaluated by the following argument. Assuming that N approaches N_s from below and that equation 10 applies when $N = N_s$, then the population density will increase after light becomes limiting only if $K/V > N_s/t_r$. However, if $K/V > N_s/t_r$ then $K t_r/V > N_s$, $(N_s - K t_r/V) e^{-t/t_r}$ is negative, and N is initially less than $K t_r/V$. As time since light became limiting increases e^{-t/t_r} becomes smaller the magnitude of $(N_s - K t_r/V) e^{-t/t_r}$ decreases in comparison with $K t_r/V$. Thus the population density in a light limited culture will approach the value Kt_r/V asymptotically. After the culture has been operating for several retention times $(N_s - K t_r/V) e^{-t/t_r}$ may be neglected in comparison with $K t_r/V$ and

$$N = \frac{K t_r}{V} \qquad 12.$$

The value Kt_r/V may be defined as the equilibrium population density in a light limited continuous algal culture.

On the other hand it is conceivable that K/V could be less than N_s/t_r. If this happens equation 10 indicates that as soon as the population reached N_s then dN/dt would be negative, the population would decrease, and light would no longer be limiting. Under these conditions it would be expected that N would oscillate around some value slightly less than N_s and no true equilibrium could be established. This phenomenon would presumably be expected if the retention time in the culture were made short enough. However, at extremely short retention time $G < 1/t_r$ and the algal population would never become established in the culture.

According to equation 12 the equilibrium population density in a light limited continuous algal culture is directly proportional to the retention time. This seems reasonable because in the light limited condition the number of new cells produced is proportional to the amount of light absorbed (57); thus the longer the medium is exposed to the light the more new cells are produced and the greater the population density.

The rate of production in a continuous culture may be defined as the population density achieved divided by the retention time or, for a light limited continuous culture of algae,

$$P = N/t_r = K/V \qquad 13.$$

This leads to the conclusion that, in a light limited culture, the equilibrium production rate is independent of the retention time. The expected variation in equilibrium population density and production rate with retention time in a light limited culture is shown graphically in figure 8.

MATERIALS AND METHODS

The primary objective of the experimental part of this investigation was the qualitative rather than quantitative verification of the theoretical predictions on the population density and production rate of algae cultured under CO_2 and light limiting conditions. Accordingly, there has been no attempt to determine any maximum or minimum values of algal concentration and yield under various environmental conditions but rather to verify the general validity of the theory under environmental conditions where the only variable was the retention time in any one series of experiments. The experiments were divided into two parts; viz. 1) continuous cultures in which carbon dioxide was the factor limiting growth and 2) continuous cultures in which the growth rate was limited by the incident light intensity. In the first series of experiments the exclusion of bacteria from the growth tube was of primary importance, since bacteria living on the metabolic by-products of the algae and producing CO_2 can alter considerably the actual amount of CO_2 available to the algae. The first series therefore was carried out with pure cultures.

In the second series the maintenance of pure cultures was desirable but not compulsory, since CO_2 was supplied in excess.

Apparatus

The apparatus consisted of two culture units operating in parallel but independently with respect to the rate of feed. Temperature and light intensity are the same for each culture in any one series of experiments. The cultures are fed by different reservoirs of media but from the same gas source.

A diagram of one of the culture units is shown in figure 9. The five main components were the culture vessel, the medium feeding system, the effluent system, the aeration system and the innoculation system. The culture vessel consisted of the culture tube, which had a volume of about one liter and contained a glass feeding tube, a thermometer, and an innoculation tube, all inserted through a rubber stopper in the top of the tube. The culture vessels were placed between two vertical boards each of which supported six, 40 watt, soft white fluorescent lamps. The two rows of lamps were 24 cm apart and 9 cm from the culture tubes. The light intensity could be varied by addition or removal of lamps and by changing the geometry of illumination. The interior face of the board was covered with aluminum foil for the reflection of light. During the experiments the cultures were continuously illuminated.

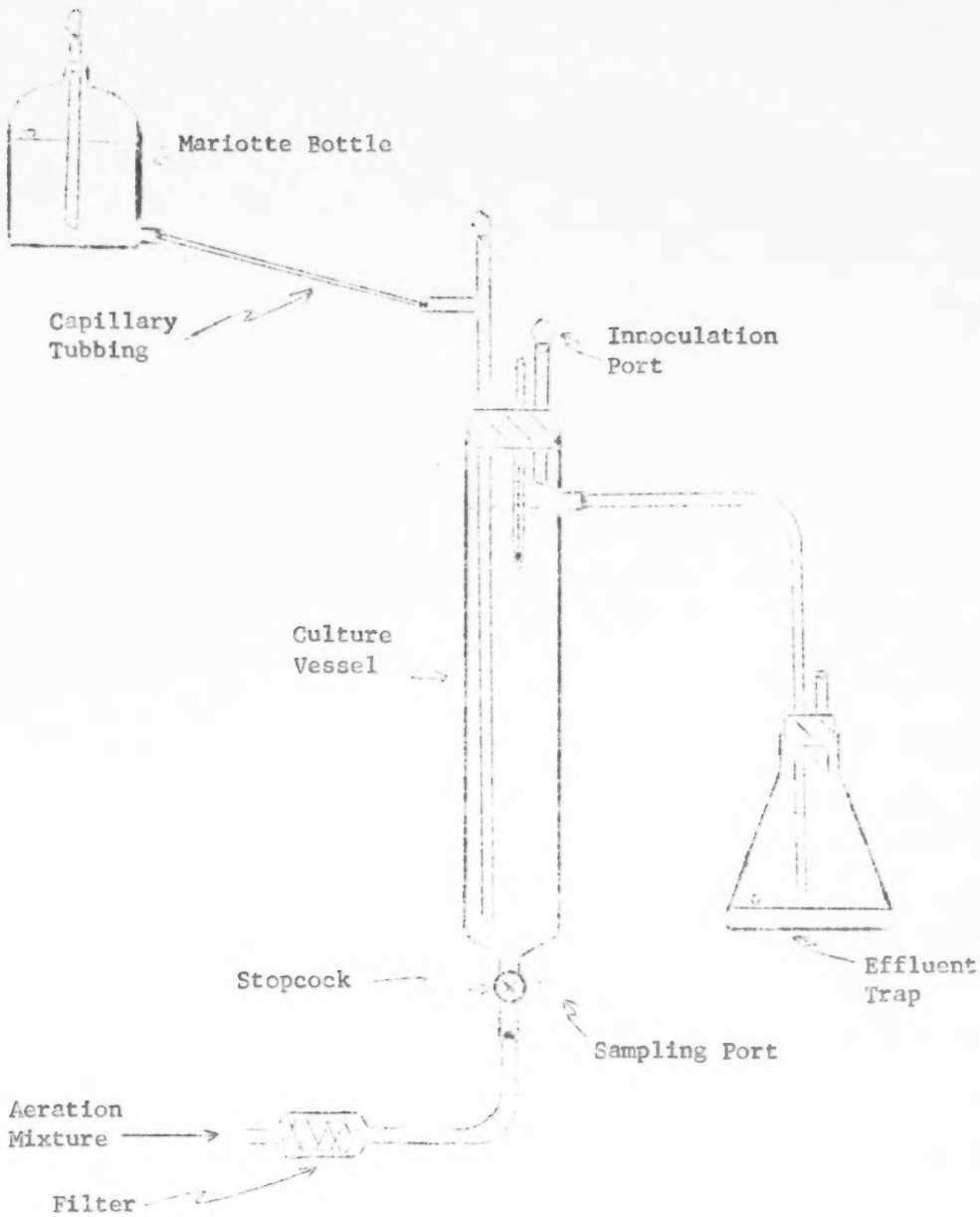

Fig. 9. Diagram of continuous culture apparatus.

The temperature of the cultures was held constant by a continuous draft of air-conditioned air passing through the two vertical boards while the temperature of the room was held constant (20°C). The temperature could be varied by deviating the draft of the air-conditioned air. During the any one series of experiments the observed variation in the temperature of the cultures was $\pm$ 1.5°C.

The medium feeding system consisted of a 4-liter Mariotte bottle containing the medium with a 1 mm I.D. capillary tube outlet to the culture. The vertical distance between the end of the capillary tube and the lower end of the air inlet in the bottle controlled the rate of flow. By varying this vertical distance, the flow rate could be varied from less than 100 ml/day to more than 1000 ml/day, and held constant to the desired value irrespective of the lowering of the level of the medium in the feeding bottle. The effluent system consisted of a 500-ml Erlenmeyer flask collecting the effluent from the culture during feeding, a gas outlet, through which the effluent gas escaped.

The aeration system consisted of a gas source (air with different percentages of CO_2 compressed in standard cylinders), a 1-liter Erlenmeyer flask containing 10^{-3}M $K_2 Cr_2 O_7$ solution, and a 4-liter glass bottle containing distilled water which both were used to scrub and humidify the gas mixture. Before entering the growth tube, the gas was passed through a cotton filter which prevented bacteria contained in the gas from entering the culture.

Medium

The medium used in these experiments was entirely inorganic; its composition is indicated in Table XI. The concentration of the major nutrients as well as the specific salts used, falls within the variations used by other investigators (4). Although the use of a chelating agent such as EDTA was desirable, it was not included into the medium because it is believed that algae grown under CO_2 limiting conditions might use EDTA as a carbon source. The small precipitate which was formed on the bottom of the medium bottle during autoclaving was resuspended by shaking the feeding bottle periodically.

Experimental Procedure

The inorganic medium was prepared from stock solutions of the corresponding components and distilled water. After the feeding bottle was filled with medium and clamps were connected, the whole culture unit was autoclaved. All the connections, air inlets, air outlets, and rubber stoppers, were wrapped with clean cotton fastened in place with strips of electrical tape. Moreover, all these "weak points", i.e., points through which bacteria could possibly find access into the unit, were covered with aluminum foil which protected the cotton from getting wet during autoclaving. The unit was auto-

claved for 45 minutes to 1 hour under 15 p.s.i. pressure and 248°F temperature. This laborious and cumbersome procedure was, by experience, found necessary to reduce the possibilities of contamination.

The sterilized unit was placed in the housings, the cotton filter was connected with the gas supply and enough medium to fill the culture vessel was allowed to pass from the bottle to the culture tube. The innoculating culture had a volume of 10 ml and was prepared by transferring a small amount of algae by an innoculating needle from stock cultures grown on agar slants. The test organism employed was the strain of Chlorella pyrenoidosa previously described in this report.

The culture was allowed to grow without any other medium feed. After 3-4 days growth was observed visually, and feeding was started. The effluent was examined every day microscopically, and if any bacterial growth was observed, the experiment was terminated.

Measurements

Determinations of pH, alkalinity, and packed cell volume of the effluent from each culture were made daily. The packed cell volume determination was made as previously described. When the packed cell volume, pH, and alkalinity of any culture had been constant for three successive days it was assumed that the culture was in equilibrium.

Light intensity was also measured whenever an equilibrium was reached in the growth vessel. An illumination meter was used to

TABLE XI

INORGANIC MEDIUM USED IN CONTINUOUS CULTURES

Nutrient	Salt	Conc. of Salt	Conc. of Nutrient
N	KNO_3	722 mg/l	100 mg/l
P	K_2HPO_4	56	10
	KH_2PO_4	44	10
Mg	$MgSO_4$	75	15
Fe	$FeCl_3.6H_2O$	6.6	1.0
Ca	$CaCl_2$	1.4	0.5
B	H_3BO_3	2.86	0.5
Zn	$ZnNO_3.6H_2O$	1.8	0.5
Mn	$MnSO_4.H_2O$	1.54	0.5
Mo	MoO_3	0.75	0.5
Cu	$CuSO_4$	1.24	0.5
Co	$CoCl_2.6H_2O$	2.00	0.5

pH = 6.6 - 6.8
Alkalinity = 15 - 20

measure both the incident light (light target in contact with the growth vessel facing the lamps) and the emitted light (light target in contact with the growth vessel facing the culture).

TABLE XII

DATA FROM CO_2 LIMITED CONTINUOUS ALGAL CULTURES

Culture Number	V (ml)	w (ml/day)	t_r (days)	N (ml/l)	pH	Alk (mgl/l)	Gas Rate (ft^3/hr)	CO_2 in Gas (%)
I-1	1000	320	3.12	1.00	7.4	66	1.0	0.03
I-2	1000	250	4.00	1.10	7.5	80	1.0	6.03
I-3	1000	200	5.00	1.50	7.7	95	1.0	0.03
I-4	1000	500	2.00	0.65	7.1	53	1.0	0.03
II-1	1000	440	2.27	1.05	8.9	74	1.5	0.03
II-2	1000	330	3.03	1.35	9.0	88	1.5	0.03
II-3	1000	300	3.33	1.55	9.1	98	1.5	0.03
II-4	1000	550	1.82	0.80	9.7	57	1.5	0.03

RESULTS

Cultures with CO_2 as the Limiting Factor

During this series air containing 0.03% CO_2 was used as the carbon source and was supplied at rates of 1 ft^3/hr and 1.5 ft^3/hr to cultures I and II respectively. The temperature of the cultures was held at 27°C and the incident light intensity was 1800 foot candles. The light intensity emitted from each culture was measured periodically and at no time during these experiments did any culture reduce the light intensity to less than 400 foot candles. Since 400 foot has been used as the light saturation value for Chlorella by other investigators (57) it is believed that in each culture in this series of experiments all cells were receiving light intensity at the saturation level or greater.

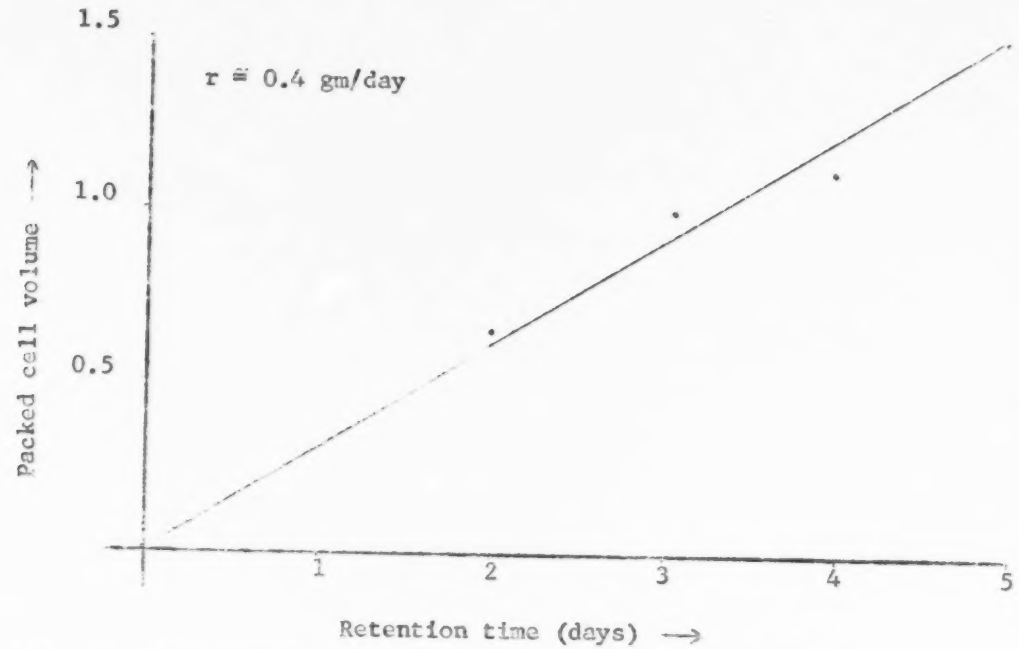

Fig. 10. Equilibrium population density in culture I.

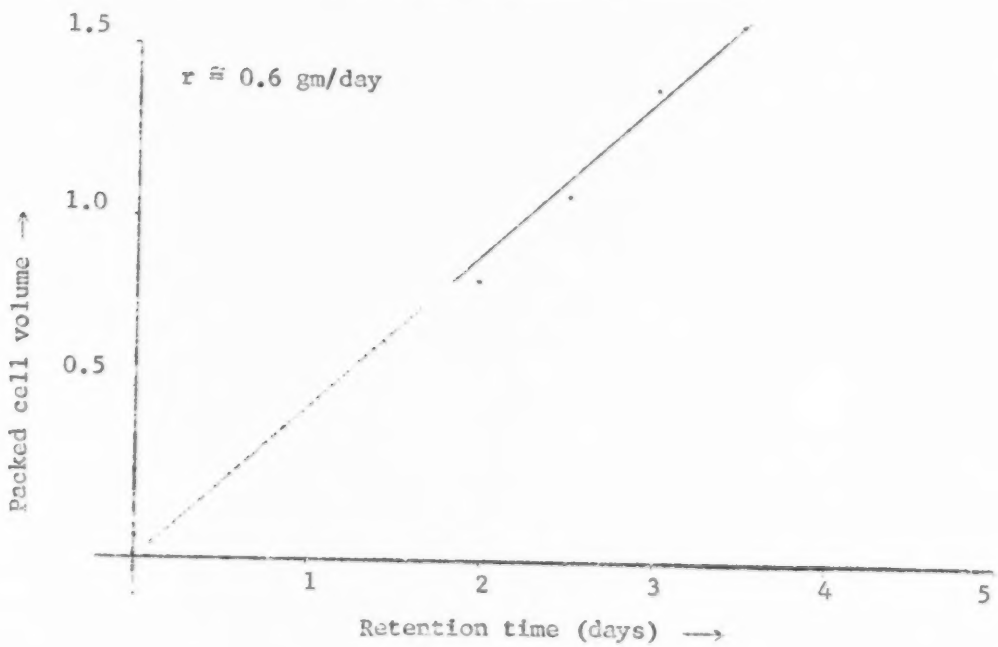

Fig. 11. Equilibrium population density in culture II.

Phosphorus Sources

In all of the reported investigations of algal nutrition and physiology phosphorus was supplied to the cultures in the form of orthophosphate (4) and there have been no experiments specifically designed to demonstrate the utilization of other forms of phosphorus by the organism. It has been reported (25) that adenosine triphosphate does stimulate the growth of Chlorella which should indicate that it is available as a source of phosphorus.

Sulfur Sources

The study of the sulfur requirements of algae has been generally neglected probably because the sulfate ion is an excellent source of sulfur and it is present in practically all culture media. Shrift (26) found that the concentration of sulfate in the medium had no effect on the growth rate of Chlorella vulgaris in the range of from 10 mg/l. to 1280 mg/l. At concentrations of 2560 mg/l. and greater the cells clumped together. He also reported that Chlorella could grow with D- or L-methionine as the sole sulfur source but that cystine would not satisfy the sulfur requirement. Apparently Chlorella cells would grow for a time with cysteine as the sole source of sulfur but the appearance of hydrogen sulfide in the medium killed the cells after a short period.

Trace Elements

It has been known for some time that all plants require various metallic ions in concentrations of 0.1 to 10 mg/l. for growth and many of the same ions are toxic at higher concentrations (27). The

They will include low molecular weight compounds that have one functional group and polyfunctional compounds of higher molecular weight such as amino acids, sugars, di- and tribasic acids, keto acids, hydroxy acids, polyalcohols, etc.

Compounds which may be extracted from a neutral aqueous solution by ethel ether but are basic enough to form salts in hydrochloric acid solution will appear in fraction A. The majority of these compounds will be amines. It is well-known that a number of amines are produced during the decomposition of proteins and therefore some quantity of these compounds would be expected to be present in sewage.

Fraction I should contain compounds that have no marked acid or basic properties. Included in this group will be fats, hydrocarbons, aldehydes, ketones, and alcohols. It would not be expected that any of these types of compounds would accelerate the growth of Chlorella.

In the process of extracting the ether solution of sewage compounds with a sodium hydroxide solution it was found that an amorphous precipitate was formed. Apparently this precipitate was either soap, detergent, or the product of the soponification of some fats that were extracted from the sewage. It was retained with the aqueous layer throughout the rest of the extraction procedure but showed no tendency to redissolve in either the aqueous solution or in the ether. It was finally separated by filtration and labeled fraction P.

The organic compounds which are acid enough to be more soluble in sodium hydroxide solution than in ethyl ether were divided into two fractions; namely, those that will form salts in sodium bicarbonate

material in the fractions were again diluted with the inorganic medium until their concentrations in the medium were approximately equal to their concentrations in the sewage. The experiments whose results are reported in Table VIII were carried out in cultures in which the concentration of carbon dioxide was inadequate to produce the maximum rate of photosynthesis and thus any acceleration of the growth rate may be

TABLE IX
TESTS OF SEWAGE ORGANICS IN PURE CULTURES
WITH ADEQUATE CARBON DIOXIDE

Material	Control	Dilution 1:0	1:10	1:100
		Growth Rate		
Sewage	1.370	1.870	1.580	1.490
Urine (diluted 1:10)	1.385	2.240	2.080	1.880
Fraction W	1.390	1.680	1.520	1.410
Fraction A	1.370	1.360	1.370	1.385
Fraction I	1.420	1.120	1.240	1.320
Fraction B	1.360	1.470	1.380	1.395
Fraction P	1.380	1.310	1.360	1.420
Fraction C	1.390	1.370	1.410	1.360

interpreted in terms of the assimilation of organic carbon. From these results it is again apparent that the compounds present in sewage that may serve as carbon sources for Chlorella are either more soluble in water at a neutral pH than in ether or form salts in basic solution. It is likely that the same compounds are responsible for the activity of fraction B and fraction C since the difference in solubility in hydroxide and in bicarbonate solution of many organic compounds is not clear cut.

It has been known for some time that many of the algae, notably Chlorella and Scenedesmus, which are frequently found in stabilization ponds, are able to utilize a variety of organic compounds as carbon sources, nitrogen sources, or energy sources when grown under the proper conditions (2). More recently it has been reported that a strain of Chlorella pyrenoidosa was able to reduce the concentration of organic matter in sterilized sewage when it was grown in continuous cultures in the absence of bacteria (3).

Thus, it would appear that some of the algae in a stabilization pond would be able to utilize some of the organic matter present directly if the environmental conditions in the pond were right. Whether or not this actually occurs in an operational stabilization pond and what effect it would have upon the growth of the algae has not been established previously.

This investigation was a study of the effect of some organic materials, both pure organic compounds and organic matter extracted from sewage, on the growth rate of an alga which commonly occurs in stabilization ponds. It was designed to provide information which might indicate if stabilization pond algae do actually assimilate organic matter from waste waters, the growth conditions under which this assimilation might take place, and what effect it would have on algal growth.

LITERATURE REVIEW

Algal Nutrition

In general, an organic compound which is utilized by a photosynthetic organism may serve in one or more of several different roles in the metabolism of the organism (4). It may serve as an oxidizing agent in the photosynthetic reaction. It may serve as a source of carbon or of a specific organic structure required by the anabolic reactions of the organism. It may serve as a source of nitrogen, phosphorus, sulfur, or another element required by the organism. It may act as a growth factor, in which case the organism will not be able to grow and reproduce in the absence of the compound. Finally, it may act as a growth substance which will accelerate the rate of cell division when present in low concentrations and inhibit growth in higher concentrations.

Carbon Sources

It has been demonstrated that Chlorella and other unicellular green algae are able to grow in media that contain only inorganic compounds and thus are not dependent upon any organic carbon source or growth factor (4). Carbon dioxide may serve as the sole carbon source, being converted by photosynthetic reactions into organic compounds that are then available to be oxidized for the release of energy. Myers (4) has presented data to demonstrate that the rate of production of organic compounds by photosynthesis in algal cells

grown under optimum conditions is greater than the rate at which the compounds are assimilated. In other words, the rate of growth in an algal culture, not limited by some external chemical or physical factor, is limited by the rate of cellular metabolism rather than the rate at which carbon dioxide is fixed by photosynthesis. According to this theory the addition of an organic carbon source to a culture in which the photosynthetic rate is maximum would not result in acceleration of the growth rate. However, it does not rule out an increase in the growth rate due to the presence of an organic compound which fills some other role in the metabolism of the alga.

In a natural body of water, or a algal culture, any carbon dioxide present will be in equilibrium with a carbonate-bicarbonate buffer system, the proportion of each form present depending upon the pH of the solution and the partial pressure of the carbon dioxide in the gases in contact with the water. It has been shown that many aquatic plants are able to utilize bicarbonate ions directly in the photosynthetic reactions but that it is doubtful that carbonate ions are utilized (5). Osterlind (6) found that Scenedesmus quadricauda is able to produce organic matter as rapidly from bicarbonate as from carbon dioxide. He also was able to demonstrate that Chlorella pyrenoidosa is unable to utilize bicarbonate ions directly in photosynthesis (7).

The effect of the concentration of carbon dioxide in the medium on the rate of growth and photosynthesis of algae is still somewhat in doubt and the optimum concentration is not firmly established.

in which vegetables are boiled is usually wasted down the drain and should contain various sugars, starches, and vitamins. As home garbage grinders are being used more and more extensively it is evident that sewage will contain fruit and vegetable peelings, table scraps, and most of the other materials which were formerly disposed of with solid wastes.

Human excretory products undoubtedly vary widely from person to person but as sewage is an integrated sample from many people the average values for the excretion of certain substances should be a useful approximation of what will be found in sewage. The Handbook of Biological Data (46) lists the following excretory products for the adult human in units of mg. per day per kilogram of body weight: creatin, 15; hippuric acid, 8; urea, 30; uric acid, 9; citric acid, 10; formic acid, 1.3; indole-acetic acid, 0.05; lactic acid, 40; oxalic acid, 0.3; and ascorbic acid, 0.38. The nitrogen excreted daily amounts to 160 mg/kg. of which 2.5 mg. is in the form of free amino acids. In addition, many other compounds have been detected in human urine and fecal matter in smaller amounts including androgens, estrogens, vitamins A, D, E, and K, phenols, biotin, etc.

If it is assumed that the average human weighs 60 kilograms and that the sewage flow amounts to eighty gallons per capita per day, then from the above data the concentrations of the various components in sewage would be: creatinine, 2.9 mg/l.; hippuric acid, 1.6 mg/l.; urea, 6 mg/l.; uric acid, 1.7 mg/l.; citric acid, 2.0 mg/l.; formic

acid, 0.2 mg/l.; indoleacetic acid, 0.007 mg/l.; lactic acid, 8 mg/l.; oxalic acid, 0.04 mg/l.; and ascorbic acid, 0.05 mg/l. These values are very rough estimates and are actually of little value except for the estimation of the maximum amounts that are likely to be found in sewage. Sewage is subjected to bacterial action for some time before it reaches the sewage treatment plant or before a sample could be taken and many of the compounds originally present will be removed by bacterial assimilation.

From the above speculations it is apparent that several of the compounds that are known to serve as nutritional substrates for Chlorella may be expected to be present in sewage. However, whether or not they are present in concentrations that would affect the rate of growth of the algae cultured in sewage ponds would be hard to determine on the basis of speculation alone.

MATERIALS AND METHODS

Test Organisms

The test organism employed in this investigation is a species of Chlorella pyrenoidosa isolated from experimental pilot plant stabilization ponds at the University of California by Dr. Mary Belle Allen in 1953. During the course of the experiments this strain of Chlorella was propogated by growth on slants of sewage agar. The transfers were made using sterile technique and the occasional cultures that were contaminated with bacteria or fungi were discarded. The experimental cultures were innoculated directly from the agar slants.

Bioassey Procedure

Certain pure organic compounds which were obtained from commercial sources and organic materials extracted from sewage were tested to determine their effect on the growth rate of Chlorella under different conditions. The pure organic compounds which were tested were selected to include representatives of several types of compounds which would be expected to be found in sewage and of the many substances that are known to serve as nutritional substrates for microorganisms or that accelerate the growth rate of other plants.

Growth Rate

The bioassey procedure employed was to determine the growth rate of the test organism on an inorganic medium and, at the same time, the growth rate in cultures to which the test material had been added. The growth rate was determined in terms of a growth constant K_g given by the formula:

$$K_g = (1/t)(\log V_t - \log V_o)$$

where t is the time of the test period, in days, V_o is the packed cell volume in the culture at the beginning of the test period, and V_t is the packed cell volume at the time t.

Medium

For the purposes of the tests it was desired to have a medium which would supply all of the necessary nutrients for the growth of Chlorella in an inorganic form. There are several media which have been used for the cultivation of Chlorella and apparently allow the

solution and those that do not. The compounds that are soluble in hydroxide but not in bicarbonate solution are mainly phenolic compounds and will appear in fraction B. The compounds which are acid enough to be soluble in bicarbonate solution are mostly carboxylic acids. They will appear in fraction C. Some compounds of intermediate acidity will appear in both fractions.

The various fractions obtained by the extraction process were tested to determine their effect on the growth rate of Chlorella. Those fractions which showed evidence of containing some compound or compounds that had an effect on the growth rate of the alga were examined further by chromatography in an attempt to determine the identity of the substances. The specific procedure was as follows: if the fraction was in an aqueous solution the pH of the solution was adjusted to neutrality and the organic compounds were extracted with ether; a few milliliters of the ether solution was used to produce a spot on a strip of Whatman #4 filter paper; the solvent used to develop the chromatogram was placed in the bottom of a tall glass cylinder and the strip of filter paper was arranged so that it hung vertically in the cylinder with the end nearest the spot dipping into the solvent. The chromatogram was allowed to develop for about 18 hours and then tested for the presence of certain suspected compounds by determination of the ultraviolet fluorescence and the reaction to certain spot tests.

The chromatograms were cut into sections so that ten sections of equal length were obtained from each chromatogram. The compounds from

each section were eluted by soaking the paper in distilled water and each solution so obtained was tested for the presence of compounds that effected the growth rate of Chlorella.

RESULTS

Preliminary Experiments

A series of preliminary experiments was undertaken to determine the range in which the concentrations of the inorganic constituents of the basic test medium had no effect on the growth rate of Chlorella pyrenoidosa. The cultures were innoculated with pregrown cells so that the initial concentration of algal cell material was about 0.20 ml/l. No attempt was made to prevent contamination of the cultures but since no organic material other than that excreted by the algal cells was present in the cultures contamination with bacteria was slight. The results of these experiments are given in Table II. The growth rate is expressed in terms of the growth constant, K_g, calculated using natural logarithms.

The results of the cultures which were supplied with adequate carbon dioxide are reported in Table IX. The only definite acceleration of growth occurred in the sewage itself and in the fraction containing the water soluble compounds. The acceleration was greater in the whole sewage than in the sewage which had been extracted with ether, possibly indicating that some but not all of the active materials had been removed in the ether.

Included in these two series of experiments were two tests of the effect of fresh urine on the growth rate of Chlorella pyrenoidosa for the purpose of comparison. Since a preliminary experiment indicated that Chlorella pyrenoidosa would not grow in fresh urine the sample was diluted 1:10 with the inorganic medium before any of the cultures were made up. Thus the dilutions indicated in the table should be divided by ten to give the actual dilution. Urine gave good acceleration of growth in all cultures in which it was tested. The urine present in sewage will be diluted about 1:400. The tests indicated that at a dilution of 1:1000 urine stimulated the growth of Chlorella as much as did raw sewage alone.

Results of Chromatography Experiments

Of all the chromatograms that were run the only one on which a substance that accelerated the growth rate of Chlorella either in the presence or absence of adequate carbon dioxide was the chromatogram of fraction C. The amount of material used for the chromatogram was equivalent to about ten liters of sewage. Since all of the compound eluted from the chromatogram was used in the test culture the concentration of

the compound in the culture was about twenty times that of the compound in the sewage. In the culture in which the carbon dioxide concentration was about 1.0 mg/l. the growth rate of the test culture was 0.424 versus 0.375 in the control. In the culture provided with adequate carbon dioxide the growth rate was 1.960 versus 1.410 in the control.

The fraction of the chromatogram showing activity had an R_f value, the ratio of the distance that the compound migrated to the distance that the front moved, of about 0.90 when the chromatogram was developed with a 10:1:1 mixture of isopropanol, ammonia, and water. It had a blue fluorescence under ultraviolet light. When moistened with a solution of 50 parts of 5% perchloric acid and 1 part of 0.5 M. ferric chloride solution it produced a pink color. After treatment with a 2% solution of para-dimethylaminobenzene in 1.2 N. HCl it developed a greenish-blue color overnight. Since all of these tests agree with the reactions of indoleacetic acid (30) the compound was tentatively identified as indoleacetic acid.

DISCUSSION

The results reported herein and those reported by other investigators indicate that a variety of organic compounds have an effect upon the growth of unicellular green algae such as are found in stabilization ponds. However, these organic compounds play various roles in the nutrition and metabolism of these algae.

Growth Limited by Nutrient not in Feed

Probably the most significant example of a continuous culture in which the limiting factor is a nutrilite supplied at a rate independent of the rate of medium feed would be an algal culture in which the limiting factor is carbon dioxide supplied by aeration of the culture. However, in the same category would be a continuous culture of aerobic microorganisms in which oxygen supplied by aeration is made the limiting factor. For such a culture, equations 1 and 2 should hold, but the mass balance for the limiting nutrilite would be signtly different. If the rate of supply of the limiting nutrilite is r then the mass balance may be expressed as

$$\frac{dc}{dt} = \frac{r}{V} - \frac{c}{t_r} - \frac{kN}{q} \qquad 7.$$

Equations 1, 2 and 7 form a system of equations which, when solved, would give expressions relating the equilibrium population density and concentration of limiting nutrilite to the retention time, rate of supply of limiting nutrilite, and time of operation of culture. The differential equation 7 is entirely analogous to equation 3 and this system of equations may be solved by the method Moser (55) employed for the solution of the system of equations 1, 2 and 3. This solution, which will not be detailed here for the sake of brevity, proves that there is a stable equilibrium condition for this type of continuous culture. At equilibrium dc/dt must be equal to zero and

$$N = q\left(\frac{rt_r}{V} - \frac{c_1}{G\ t_r}\right) \qquad 8.$$

Equation 8 is actually a more general form of equation 4 as may be seen by substituting r = aw into the expression. The production rate in this type of culture would be given by

$$P = N/t_r = q\left(\frac{r}{V} - \frac{c_1}{G\ t_r^2}\right) \qquad 9.$$

From equation 8, it may be seen that the equilibrium population density in this case will increase with increasing retention time and at the greater values will be directly proportional to it since $c_1/G\ t_r^2$ becomes negligable. The equilibrium production rate will be independent of t_r when N is directly proportional to it. The expected variations of population density and production rate at equilibrium in a continuous culture in which the limiting nutrilite is added at a constant rate independent of the medium feed rate are shown graphically in figure 7.

Growth limited by Light Intensity

Tamiya and his co-workers (57) observed that when a batch culture of an alga is started with a small innoculum, so that only a small part of the incident light is absorbed, and the algae are allowed to grow under constant incident light intensity and constant temperature, growth generally proceeds in two distinct phases. During the earlier phase d(logN)/dt remains constant; while during the second phase, dN/dt becomes constant. Both of these constant values depend upon light intensity. This transition from the exponential to the linear phase occurs at different population densities, depending upon the incident light intensity. Above the critical density growth in a

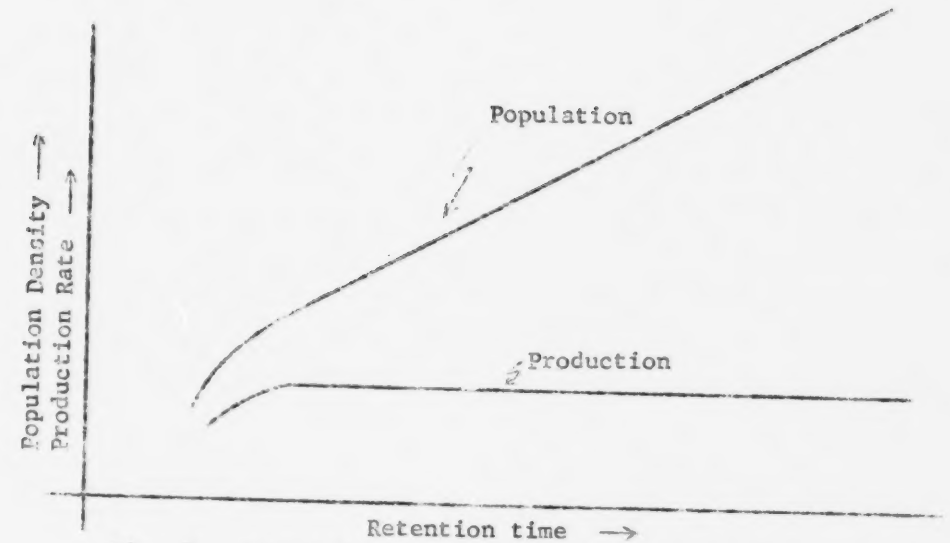

Fig. 7. Expected variation of population density and production rate in a CO_2 limited continuous algal culture.

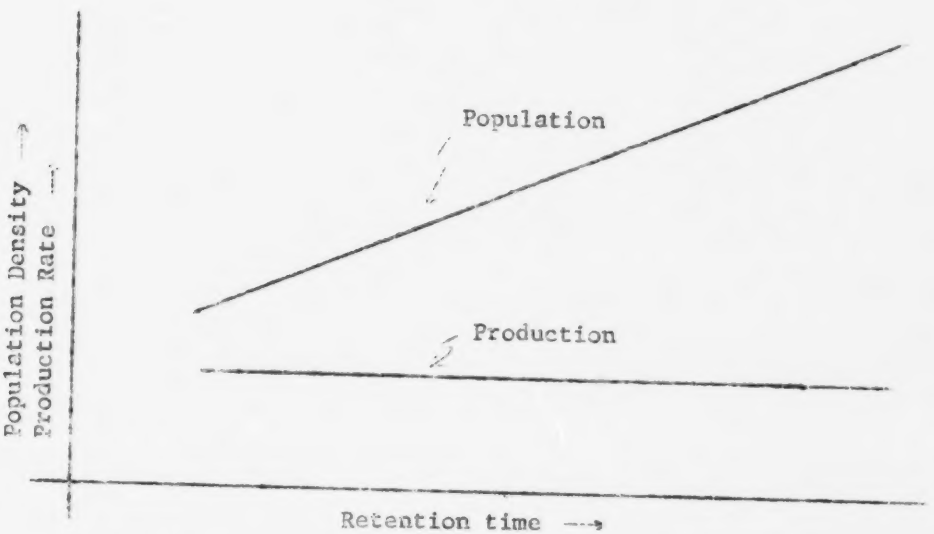

Fig. 8. Expected variation of population density and production rate in a light limited continuous algal culture.

The results of these series of experiments are presented in Table XII. The value of the population density, N, for each culture was constant for at least three days before it was concluded that the culture was in equilibrium. In some cultures minor fluctuations in the flow rate, w, occurred during the period in which the culture was in equilibrium, however, in no case was the fluctuation in the retention time greater than 5% of the average retention time while the culture was in equilibrium.

Figures 10 and 11 present plots of the equilibrium population density versus retention time for cultures I and II respectively. Equation 8 predicts that the relation between the equilibrium population density will be

$$N = q\left(\frac{r\ t_r}{V} - \frac{c_1}{G\ t_r}\right) \qquad 8.$$

However, at equilibrium $k = 1/t_r$ and when c is much, much less than c_1 $c = kc_1/G$. Thus, the term $c_1/G\ t_r$ represents the concentration of the limiting nutrient. At longer detention periods the concentration of the limiting nutrient will be much, much less than $r\ t_r/V$ and equation 8 will reduce to

$$N = q\ \frac{r\ t_r}{V}$$

If q, r, and V are constant the equilibrium population density will be directly proportional to the retention time. The volume of the cultures was held constant and it has been shown q is independent of c (5). For cultures I and II the gas flow rates were 1.0 and 1.5 ft^3 per hour respectively. With 0.03% CO_2 in the influent air this is

about 400 and 600 mg/day of CO_2 to the cultures respectively. Undoubtedly some CO_2 was present in the effluent gas from each culture and the estimates of r are higher than the actual rates at which the algae received carbon dioxide. However, the variation in the rates at which the algae received CO_2 at the various retention times was not enough to influence the growth rates since the equilibrium population dencity plotted as a linear function of retention time.

The production rates for cultures I-1 through I-4 were 0.32, 0.275, 0.30, and 0.325 respectively, giving an average of 0.305. Those of cultures II-1 through II-4 are 0.463, 0.445, 0.465 and 0.440 respectively, giving an average of 0.453. According to equation 9 the production rate for these cultures should be equal to $q\ r/V$ when $c_1/G\ t_r^2$ is so small as to be negligible. The data verify the conclusion that the production rate should be directly proportional to the rate of supply of the limiting nutrient, since 0.453 is almost 1.5 times 0.305. The data also indicate that the production rate is independent of the retention time.

Cultures with Light as the Limiting Factor

A series of ten light limited cultures was run to determine the variation in population density with retention time. The cultures were illuminated from one side with light from soft white fluorescent lamps. The incident light intensity on the side facing the lamps was 200 foot candles. The temperature of the cultures was maintained at $18^\circ \pm 5^\circ C$ during this series.

TABLE XIII

DATA FROM LIGHT LIMITED CONTINUOUS ALGAL CULTURES

Culture Number	V (ml)	w (ml/day)	t_r (days)	N (ml/l)	P (ml/l/day)	pH	Alk. (mg/l)	CO_2 in Gas (%)
III-1	1000	380	2.63	0.75	0.285	6.8	80	3
III-2	1000	330	3.33	1.00	0.300	6.6	96	3
III-3	1000	500	2.00	0.60	0.300	6.5	56	3
III-4	1000	660	1.52	0.40	0.263	7.1	48	1
III-5	1000	250	4.00	1.05	0.262	7.2	94	1
IV-1	1000	350	2.86	0.70	0.245	7.0	64	1
IV-2	1000	520	1.92	0.50	0.260	6.7	56	3
IV-3	1000	500	2.00	0.55	0.275	6.7	64	3
IV-4	1000	220	4.54	1.25	0.275	7.0	106	1
IV-5	1000	270	3.70	1.05	0.284	7.2	102	1

The data obtained from this series of cultures is presented in Table XIII. Some of the cultures were aerated with air containing 1% CO_2 and others with air containing 3% CO_2. The rate of aeration in all cultures was approximately 1 ft^3/hr. It has been shown by other investigators that aeration with air containing 1-10% CO_2 provides adequate CO_2 for Chlorella (12) and since equivalent results were obtained using either 1% or 3% CO_2 in the aeration mixture it is indicated that CO_2 concentration was not limiting in these cultures.

TABLE II

EFFECT OF INORGANIC NUTRIENTS ON THE GROWTH RATE OF *CHLORELLA*

Element Tested	Source	Concentration (mg/l.)	Growth Rate (K_g)
N	KNO_3	0	0.204
N	KNO_3	1	1.310
N	KNO_3	10	1.385
N	KNO_3	100	1.410
N	KNO_3	1000	1.380
N	$(NH_4)_2SO_4$	0	0.180
N	$(NH_4)_2SO_4$	1	1.290
N	$(NH_4)_2SO_4$	10	1.390
N	$(NH_4)_2SO_4$	100	1.355
N	$(NH_4)_2SO_4$	1000	1.385
P	KH_2PO_4	0	0.245
P	KH_2PO_4	1	1.360
P	KH_2PO_4	10	1.380
P	KH_2PO_4	100	1.390
P	KH_2PO_4	1000	0.988
Mg	$MgSO_4$	0	-----
Mg	$MgSO_4$	1	0.385
Mg	$MgSO_4$	10	1.340
Mg	$MgSO_4$	100	1.385
C	1% CO_2 in air	17.25	1.420
C	air	0.52	0.348
C	K_2CO_3 and acid	35.0	1.390
C	K_2CO_3 and acid	1.5	0.364

In general, the concentration of nitrogen, phosphorus, or magnesium in the medium had no effect on the growth rate of *Chlorella* over a wide range of concentrations. Even when nitrogen or phosphorus was not supplied in the medium there was some growth, probably due to the quantities of these elements which *Chlorella* cells will accumulate and store. Apparently magnesium is not stored by *Chlorella* cells since no growth was observed in the culture in which magnesium was not present and the cells in this culture turned a light yellow indicating lack of

Some compounds such as sugars and polycarboxy acids can serve as sole carbon and energy sources. The algae are able to grow in the dark when provided with adequate amounts of these compounds and the necessary inorganic nutrients. Compounds belonging to this category would be expected to serve as additional carbon sources for algae which are photosynthesizing if either light intensity or carbon dioxide concentration is limiting the rate of photosynthesis. However, they apparently do not increase the growth rate of algae in cultures in which conditions are optimum for photosynthesis.

A second group of compounds may serve as sources of both carbon and nitrogen. To this group belong urea, amino acids, and other products of protein decomposition. Members of this group do not serve as sole substrates for algal growth in the dark. However, they do increase the growth rate compared to that attained on an inorganic medium if light is limiting, if CO_2 is limiting, or if conditions are optimum for photosynthesis. Thus it would appear that these compounds are superior to nitrate or ammonia as nitrogen sources for some unicellular green algae.

The third group of compounds which affect algal growth rates are the growth substances. These compounds do not serve as carbon or nitrogen sources and do not increase algal growth rates when some environmental factor is limiting the rate of photosynthesis. At proper concentrations they will accelerate the growth rate of algae under conditions optimum for photosynthesis. However, at greater than optimum

The variation in the equilibrium population density with retention time for this series of cultures is plotted in figure 12. A regression analysis of the data plotted indicated that the equation of the line of best fit is $N = -0.004 + 0.277\ t_r$. According to equation 12 the intercept of this line should be zero. The value of -0.004 was found to be not significantly different from zero and thus the equation of the line was taken as $N = 0.275\ t_r$. The value 0.275 is of course the average production rate as may be calculated from column six of Table XIII.

The daily measurements of the population density in culture no. III-5 are plotted in figure 13 to give a representation of the manner in which the population density in this culture approached an equilibrium value. The curve does show a decreasing rate of increase in population density as equilibrium is approached. However, no attempt was made to fit a curve to this data because the measurements of the packed cell volume were made on the effluent collected over a one day period and it would be expected that these values would be somewhat less than the instantaneous population density in the culture until equilibrium is reached.

DISCUSSION

The analytical model presented herein relates the population density attained in a continuous algal culture to the factor which is limiting the growth rate of the algae. If some nutrient in the feed is limiting, the population density should be independent of retention time. If some nutrient fed at a constant rate independent of the rate of medium

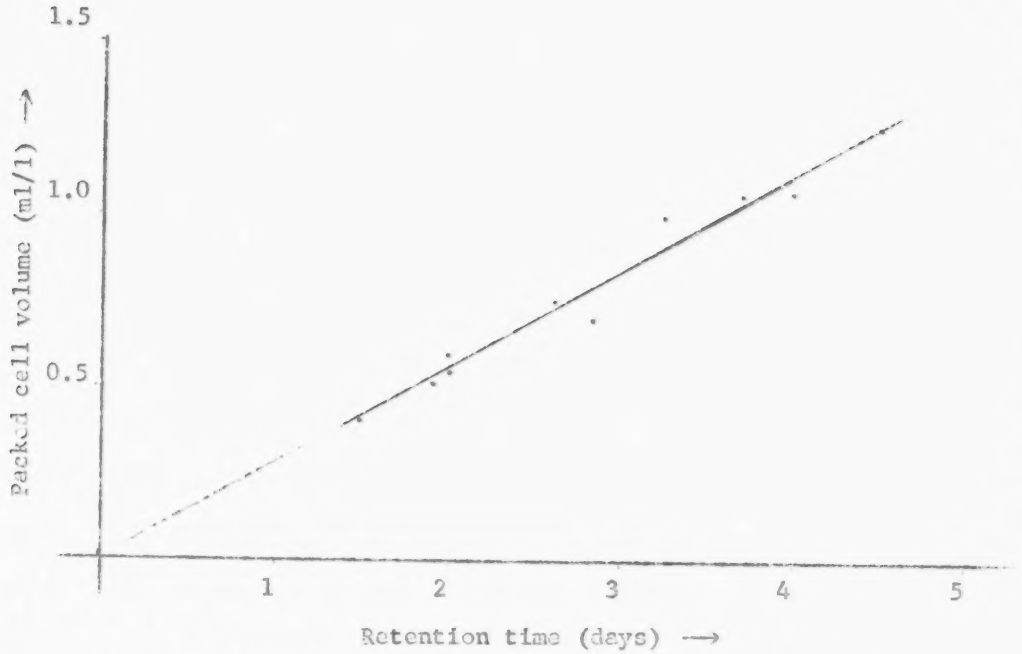

Fig. 12. Equilibrium population density in light limited continuous algal cultures.

Fig. 13. Approach to equilibrium (Culture III-5).

feed is limiting, the population density should be linearly proportional to the retention time. If the incident light intensity is the factor limiting growth the population density should also be linearly proportional to the retention time.

These relations will hold only within a limited range of retention times. At extremely low retention times the algal cells will be washed out of the culture more rapidly than they can grow and thus the equilibrium population density will be zero. At very long retention times it will be very difficult to assure that the growth rate is limited by the desired factor. The longer the retention time, the lower the flow rate and the more likely that some unsuspected nutrient in the feed will become limiting. Also if either light or CO_2 is made limiting the longer the retention time the greater the population density and the greater the average age of the algal cells. Under these conditions it is likely that either space available per cell or accumulation of waste products will become limiting.

The theory as presented is not directly applicable to stabilization ponds because it describes the growth of algae in pure cultures on an inorganic medium. In stabilization ponds organic compounds which may be metabolized by the algae are present, carbon dioxide is supplied by bacterial oxidation of organic matter, not necessarily at a constant rate, and the incident light intensity varies periodically.

The theory presented does offer a starting point for development of a model which would describe algal growth in a stabilization pond. A logical sequence for the extension of the model to more complex situations

would be 1) a study of the effect of supplying organic substances to an algal culture limited by CO_2 or light, 2) determination of the effect of periodic variation of light intensity on algal cultures, 3) analysis of the rates of oxygen production in the various types of algal cultures, 4) investigations of the rates of bacterial oxidation and CO_2 production in continuous cultures on organic media, and 5) synthesis of the overall model. Fortunately, much of the information needed for completion of the steps listed is available in the literature.

CONCLUSIONS

1. A theory describing algal growth and production in continuous cultures as a function of the factor limiting growth has been developed and verified experimentally.

2. This theory should serve as a starting point for the development of an analytical model describing algal growth in a stabilization pond.

EFFECT OF pH VARIATION UPON BOD REMOVAL IN STABILIZATION PONDS

INTRODUCTION

Speculations on the conditions of algal growth in stabilization ponds lead to the conclusion that at times the growth of the algae might be limited by the concentration of free carbon dioxide in the pond. Since the free carbon dioxide in a stabilization pond is a part of the carbonate-bicarbonate buffer system it would be expected that a decrease in carbon dioxide concentration would be accompanied by an increase in the pH of the pond. It is possible that a high pH might have a detrimental effect upon the growth and metabolism of the microorganisms in a stabilization pond.

Observations that the pH of a stabilization pond may reach values as high as 10.0 to 11.0 during the day are not uncommon in the literature (58) (59). At least one group (60) has studied the diurnal variation of pH in stabilization ponds and found that it is quite characteristic; the pH rises to a maximum in the afternoon and then decreases reaching a minimum, usually the pH of the influent sewage, at night.

Keefer and Meisel (61) found that activated sludge could be acclimated for proper operation if the pH was within the range 6.0-9.0. However,

when the pH of the influent sewage was above 9.0 the reduction of BOD was poor and above 10.0 reasonable operation was not possible. Although there are evident differencies between stabilization ponds and activated sludge it is likely that the heterotrophic organisms present in a stabilization pond will be similar to those comprising the microbial population of activated sludge in their metabolic capabilities. If this is true, extrapolation of the results obtained with activated sludge would indicate that if the pH of a stabilization pond is above 9.0 the oxidation of organic matter by the heterotrophic organisms is severely inhibited. Unfortunately, the time when the pH of a stabilization pond is above 9.0 coincides with the greatest rate of photosynthetic oxygen production.

Algae of the type which are commonly present in stabilization ponds have been grown in culture media with pH values in the range 3.0 to 11.0 with no apparent inhibition of photosynthesis or respiration (62). Oswald (63) found that the light conversion efficiency of algae grown in laboratory cultures on sewage decreases as an approximately linear function of the duration of time that the culture had a pH greater than 8.0. However, he ascribed this not to any direct effect of high pH upon the algae but to limitation of the amount of carbon dioxide available because bacterial oxidation was inhibited. In the results of another study (64) it was concluded that high pH values definately limited bacterial oxidation and resulted in increased BOD values in pond effluent.

It has been suggested previously (63) that the mechanism by which the pH of a stabilization pond is increased is a net decrease in the concentration of free CO_2 in the pond. That is, during periods when

sufficient light is available the algae remove CO_2 from solution at a faster rate than the heterotrophs give off resiratory CO_2. As the concentration of free CO_2 decreases there is a shift in the concentrations of bicarbonate and carbonate ions according to the equations

$$2HCO_3^- \rightleftharpoons CO_2 + CO_3^= + H_2O$$

or

$$HCO_3^- \rightleftharpoons CO_2 + OH^-$$

in order to maintain the equilibrium between carbon dioxide, bicarbonate ions, and carbonate ions. This hypotheses is supported by the following observations; 1.) the increase in pH occurs only during periods when light is available to drive the photosynthetic mechanism, 2.) the increase in pH is roughly proportional to the intensity of the incident light, and 3.) the pH increase may be controlled by bubbling with CO_2 enriched air (63).

However, the above hypothesis does not explain another observation; namely, that the total alkalinity of the pond contents is decreased at the same time that the pH is increased (60). According to the above equations any decrease in the concentration of bicarbonate ion is accompanied by an equivalent increase in the concentration of either hydroxide or carbonate ion so that the total alkalinity remains constant. It is possible to write a reaction to obtain CO_2 from carbonate ion:

$$CO_3^= + H_2O \rightleftharpoons CO_2 + 2OH^-.$$

However, it is doubtful that this reaction would occur and, even if it did, it would not result in a decrease in alkalinity because the decrease in the concentration of carbonate ions is accompanied by the production of an equivalent concentration of hydroxide ions.

In order to explain the decrease in alkalinity in a stabilization pond it is necessary to postulate a mechanism for the production of acid. Myers and Cramer (65) have shown that when ammonia is employed as a nitrogen source by photosynthesizing algae the consumption of one mole of ammonia is accompanied by the production of one mole of hydrogen ion. Since most of the nitrogen of sewage is present as ammonia or as organic nitrogen which is converted to ammonia by bacteria it is likely that much of the decrease in alkalinity in a stabilization pond may be ascribed to an effect of the nitrogen metabolism of the algae.

The above hypothesis; namely, that the increase in pH in a stabilization pond is due to more rapid consumption than production of CO_2 and that the decrease in total alkalinity is due to production of acid by the algae as they utilize ammonia; seems reasonable and it will check out roughly on quantitative calculations of pH, alkalinity, and ammonia concentration changes observed in a typical stabilization pond. However, it is not complete because it ignores the buffering effects of the phosphates present, the effect of precipitation of calcium carbonate, calcium phosphate, magnesium hydroxide, and magnesium ammonium phosphate at high pH values, and several known metabolic effects of bacteria on the pH of the environment.

If the oxidation of organic matter in a stabilization pond is inhibited by high pH during periods when the greatest concentration of dissolved oxygen is available and if, at the same time, the rate of photosynthetic oxygenation is decreased by low concentrations of carbon dioxide, it would be expected that better BOD removal efficiencies could be attained if the pH variation could be limited.

The most precise method of pH control for any industrial process is the positive method of continuous pH measurement and adjustment by the addition of acid or alkali. There are several reasons why this method would be unsuitable for use in a stabilization pond. In order to get pH value significant for the pond as a whole it would be necessary to employ continuous and rather violent mixing while acid or alkali were being added. The degree of mixing needed would probably make the cost of operation of a stabilization pond greater than the cost of operation other biological waste treatment processes. The control mechanisms needed for precise pH adjustment are expensive to purchase and maintain. Also precise pH control is probably not necessary since the organisms present apparently are only slightly affected by pH variations in the range 6.0 - 9.0.

Several less expensive and less precise methods of pH control could be employed for stabilization pond. One method would be to adjust the pH of the influent sewage downward during the daytime so that the pond would be continually receiving some acid to offset the tendency of pH increase in the pond. This method would require the use of acid feeding equipment, continuous monitoring of the pH of the influent and the pond and some degree of mixing in the pond.

A second feasible method would be to load the pond more heavily during the day than at night. The pond would be diluted with more sewage which has a lower pH and a higher buffer capacity than the pond contents. The concentration of putrescible organic matter in the pond would be increased and thus, if the rate of oxidation of this organic matter is proportional to the concentration of organic matter, the

rate of CO_2 production would be increased. The turbidity of the pond would be increased thus decreasing the rate of photosynthesis and the rate of CO_2 utilization. All of these effects would tend to keep the pH of the pond from increasing so rapidly or attaining so great a value.

In this investigation it was decided to study two of the methods of pH control described above, namely, 1) decreasing the pH of the influent and 2) feeding the pond only during the day. It was felt that a study of these two methods would give a basis upon which to evaluate the effect of pH control upon BOD removal efficiencies.

MATERIALS AND METHODS

The apparatus employed in this investigation consisted of four, laboratory scale, model stabilization ponds. A diagrammatic representation of the apparatus is given in figure 14. Each pond was contained in a white enamel pan and had demensions of 19" x 11" x 3" which gave each pond a surface area of 209 square inches (3.34×10^{-5} acres) and a volume of 10.3 liters (8.35×10^{-6} acre-ft).

The ponds were illuminated by either 40-watt soft white fluorescent light or by 300-watt incandescent flood lights. A combination of two fluorescent lamps at a distance of six inches above the pond surface gave an incident light intensity of about 600 foot candles. Six fluorescent lamps four inches above the pond surface gave an incident light intensity of approximately 1000 foot-candles. An average light intensity of 1600 foot-candles could be achieved using the incandescent lights. The lights were controlled by a time switch and the periodicity of light employed in all experiments was 12 hours on and 12 hours off.

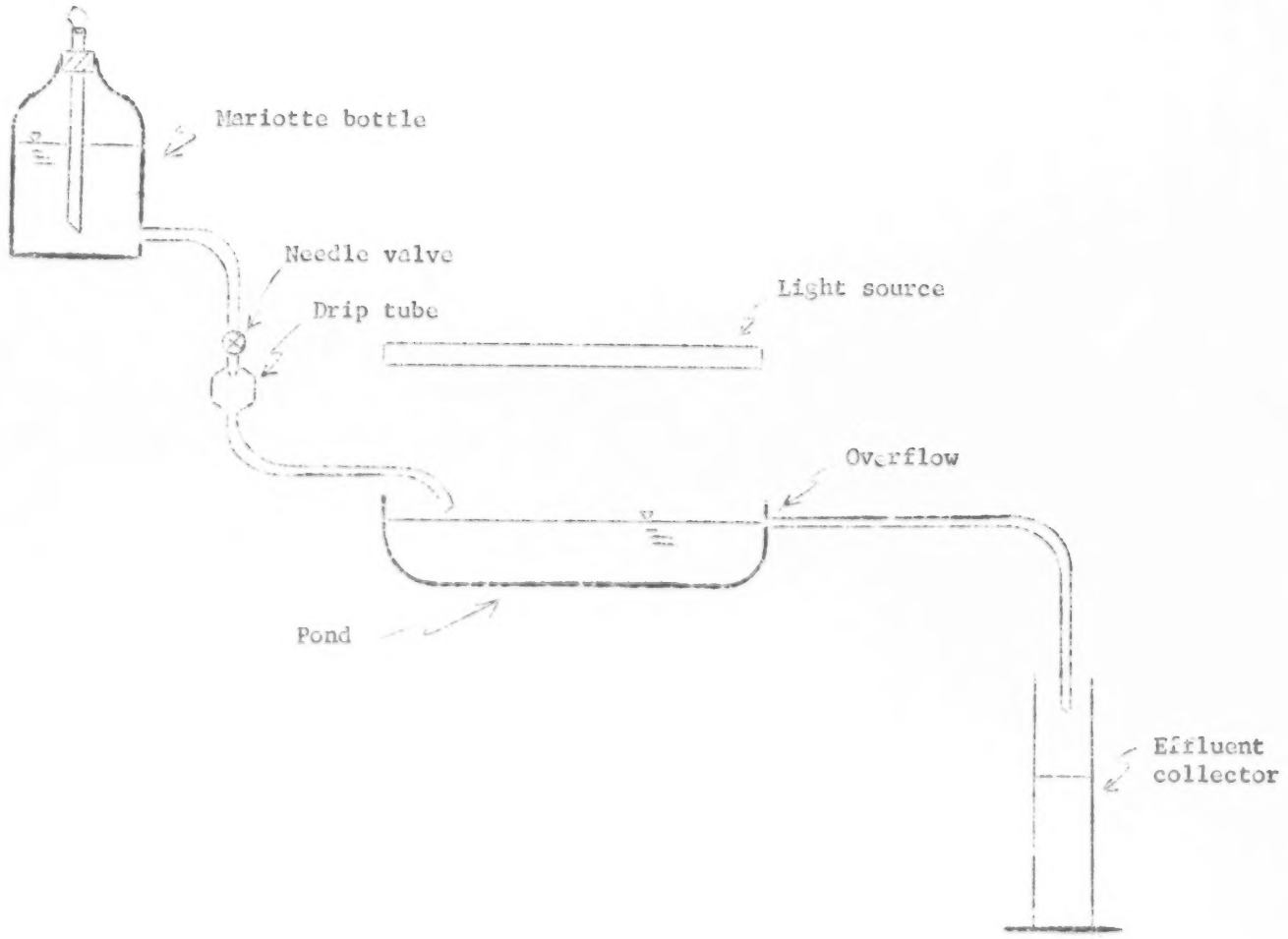

Fig. 14. Laboratory scale, model stabilization pond (diagrammatic).

Ideally the feed to the ponds should have been fresh domestic sewage. However, in order to have a constant composition feed for the duration of one set of experiments it would have been necessary to collect and sterilize a large batch of sewage. It was felt that the sterilization of the sewage would introduce changes in the composition of the organic material present and thus that the use of a synthetic sewage would produce results which were as good as could be attained using sterilized sewage.

Two different media were employed in this investigation. The composition of these two media are given in tables XIV and XV. Both media were essentially modified Knop's solution with potassium bicarbonate and organic substrate added to supply alkalinity and BOD respectively. The pH of the media was adjusted to the desired value by addition of either hydrochloric acid or potassium hydroxide before autoclaving. Thus the pH and the alkalinity of the influent medium was varied from one series of experiments to the next.

For each series of experiments the medium was made up and autoclaved in four liter Mariotte bottles. The outlet of each bottle was connected by rubber tubbing through a needle value and a drip tube to one of the ponds. The rate of medium flow to each pond was adjusted each day by means of the needle value setting. The flow was estimated by counting the number of drops in the drip tube over a period of one minute. Each of the Mariotte bottles used as a feed reservoir was calibrated so that the volume of medium remaining in the bottle could be estimated to the nearest 10 ml by measuring the depth of medium remaining.

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TABLE XIV

MEDIUM A: COMPOSITION AND ANALYSIS

Composition	
Dextrose (Difco)	180 mg/l
Beef Extract (Difco)	60 mg/l
Tryptone (Difco)	125 mg/l
KH_2PO_4	6.8 mg/l
K_2HPO_4	8.7 mg/l
$KHCO_3$	150 mg/l
$MgSO_4$	5.0 mg/l
EDTA	5.0 mg/l
Micronutrients	
Fe	0.5 mg/l
Ca	0.5 mg/l
Zn	0.5 mg/l
Mn	0.1 mg/l
Cu	0.1 mg/l
Co	0.1 mg/l
Mo	0.1 mg/l
B	1.0 mg/l
Analysis	
BOD	222 mg/l
Organic Nitrogen	18.9 mg/l
Ammonia Nitrogen	0.0 mg/l
pH	variable
Alkalinity	variable

Thus the flow into each pond over a 24 hour period could be determined by measuring the decrease in the depth of medium remaining in the reservoir at the beginning and end of that period.

TABLE XV

MEDIUM B: COMPOSITION AND ANALYSIS

Composition	
Milk solids	200 mg/1
Urea	20 mg/1
KH_2PO_4	6.8 mg/1
K_2HpO_4	8.7 mg/1
$KHCO_3$	250 mg/1
$MgSO_4$	5 mg/1
EDTA	5 mg/1
Micronutrients	
Fe	0.5 mg/1
Ca	0.5 mg/1
Zn	0.1 mg/1
Mn	0.1 mg/1
Cu	0.1 mg/1
Co	0.1 mg/1
Mo	0.1 mg/1
B	1.0 mg/1
Analysis	
BOD	160.6 mg/1
Organic Nitrogen	31.4 mg/1
Ammonia Nitrogen	0.0 mg/1
pH	variable
Alkalinity	variable

During the experiments some difficulties with rapid evaporation from the ponds were encountered. The daily evaporation from each pond ranged from about 100 ml. to almost 800 ml. In order to overcome the effect of evaporation, once each day the amount of medium

ACCUMULATION OF ALGAL SOLIDS IN STABILIZATION PONDS

INTRODUCTION

One of the features which distinguishes high rate from conventional stabilization ponds is the fact that the concentration of algae attained in the high rate ponds is much greater than those produced in conventional ponds. Concentrations of algal solids as great as 300 mg/l have been attained in pilot plant high rate ponds, and concentrations in the range 150-250 mg/l are frequently observed (1). These relatively great algal population densities are necessary for the proper operation of high rate stabilization ponds because photosynthesis is relied upon as the primary means of oxygen supply.

If the effluent from a high rate stabilization pond should reach a body of water in which the environmental conditions were adverse and the algae were killed, the organic load released from the dead algal cells would be greater than the organic load of the original waste. For this reason it has been believed that it would be necessary to remove the algae from the effluent from a high rate stabilization pond.
Also, if the algae produced in a high rate stabilization pond could be harvested, dried, and packaged at a reasonable cost, they could be sold as animal feed.

An extensive study of the possible methods of harvesting algae from high rate stabilization ponds was previously carried out at the University of California (66). This study covered such methods of harvesting as centrifugation, flocculation and sedimentation, filtration, screening, electrostatic precipitation, and removal on ion exchange resins. The results of this study indicated that not only the initial harvesting but also dewatering and drying of the algal solid would be expensive.

Normally unicellular green algae such as Chlorella and Scenedesmus are the predominant algal forms in stabilization ponds. Because of their small size these algae settle very slowly and retain large amounts of bound water. It is evident that if a high rate stabilization pond could be operated so that the predominant algal species was a filamentous form which could be harvested by sedimentation or microstraining, the cost of recovering the algae might be reduced considerably.

A preliminary search for filamentous algae which could grow in grossly polluted water resulted in the isolation of a species of Oscillatoria which grew well in domestic sewage and in a synthetic milk waste. A series of laboratory experiments indicated that when this organism was grown in laboratory cultures on sewage or the synthetic milk waste, the rates of algal production and BOD removal were comparable to those attained with Chlorella or Scenedesmus (67).

Once it was shown that this organism is able to grow well in organic wastes, the question of why it does not occur frequently in stabilization

ponds arose. Since stabilization ponds are freely innoculated with all microorganisms in the environment, the only apparent reason for the infrequent occurrence of Oscillatoria in stabilization ponds is that unicellular green algae have a competitive advantage over filamentous algae in the usual stabilization pond environment.

In a stabilization pond the algae compete mostly for nutrients and light. The preliminary studies with Oscillatoria indicated that it could derive its necessary nutrients from sewage. However, the specific growth rate, i.e., growth rate per unit mass of algal material present, of Oscillatoria was found to be less than that of Chlorella or Scenedesmus. The only method of obtaining production rates with Oscillatoria comparable to those attained with unicellular green algae was to operate with high concentrations of algae in the cultures.

In a stabilization pond any filamentous algae will settle out rapidly. The unicellular green algae which remain suspended will absorb much of the light energy which reaches the pond, and the filamentous forms will receive only a small amount of light. Thus, the filamentous algae which grow more slowly than unicellular algae anyway are at a disadvantage in a normally operated high rate stabilization pond.

An investigation was undertaken to determine if a high rate stabilization pond could be operated in such a manner that a filamentous alga could be accumulated in great enough concentrations so that it would have an over-all growth rate greater than the unicellular algae and would become predominant. The most obvious method of accumulating the

filamentous alga was to settle the effluent from the ponds for a short period of time and return those algae which settled out to the pond.

METHODOLOGY

The apparatus employed in this investigation consisted of six laboratory scale, model stabilization ponds. Each pond had dimensions of 10" x 4" x 2.75" giving a volume of approximately 1800 ml and was contained in a stainless steel pan. The pans were immersed to a depth of three inches in a thermostatically controlled water bath, and the temperature of the ponds was held to the range 15-17 $^{\circ}$C during the investigation, as this temperature range is reasonably representative of that of a stabilization pond during the summer.

A light intensity of 2500 foot candles at the surface of the ponds was produced by 3000 watt incandescent flood lamps at a distance of four inches from the ponds. This light intensity is much less than that of bright sunlight but the ponds are much more shallow than prototype ponds. The ponds were lighted for twelve hours each day.

Each pan was covered with a glass plate in order to minimize evaporation. In order to simulate the effect of surface air currents, each pond was aerated at a rate of about 0.1 cubic ft. per hour. This aeration rate gave one bubble every two to three seconds but because of the shape of the ponds very little mixing occured.

The medium employed in this investigation was the same as was used in the preliminary study of the growth of Oscillatoria on organic

wastes (67). The average analysis of eight samples of this medium is given in Table XVII. The medium has a much higher BOD than normal sewage but the high BOD was necessary to reproduce the BOD loadings of high rate stabilization ponds at reasonable detention periods.

The apparatus employed for control of the feed rate was similar to that described by Ware (68). It was planned to operate the six ponds at detention periods of 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 days respectively. However, a great deal of difficulty was experienced in obtaining stable flow rates with the feed apparatus and the flow rates varied considerably from day to day and gave nominal detention period in the range 1.0 to 10.0 for one-day periods. However, this was not thought to be too disadvantageous because in actual stabilization ponds the influent flow, and thus the detention period, may vary over a wide range.

The ponds were originally innoculated with the strain of Oscillatoria isolated from sewage and with strains of Chlorella and Scenedesmus previously isolated from stabilization ponds. The effluent from each pond collected over a twenty-four hour period was well mixed and allowed to settle for a half hour. The supernatent was then decanted and the algal solids which had settled out were returned to the pond.

The effluent from each pond was removed through an overflow device, thus, any algae leaving with the effluent were in suspension and the algae which had a tendency to settle out in the pond were preferentially retained. This method of operation combined with the return of the algal solids which settled out of the effluent was intended to provide a competitive advantage to the filamentous algae.

TABLE XVII

MEDIUM ANALYSIS

Constituent	Concentration	
Total Solids	1,166	mg/l
Ash	88.8	mg/l
Dissolved Solids	1,094	mg/l
Total Nitrogen	57.2	mg/l
Organic Nitrogen	55.2	mg/l
Ammonia Nitrogen	2.0	mg/l
BOD	650	mg/l
Alkalinity	160	mg/l @ $CaCO_3$
pH	6.9	

Since algal solids were being returned to the ponds, an equilibrium condition in respect to the concentration of algae present was never attained. Once each week the total solids and suspended solids concentrations of the pond contents were determined. The Gooch crucible procedure was employed for the determination of suspended solids. Microscopic examination of the pond contents indicated that zoogleal flocs were not present, and the results of the suspended solids determinations were interpreted to be a measure of the concentration of algal solids which could easily be removed from the effluent by filtration.

The filtrates from the suspended solids determinations were used for 5 days, $20^{\circ}C$ BOD determination. Since these filtrates contained some quantities of unicellular green algae and the BOD samples were incubated in total darkness, the values for effluent BOD are probably somewhat high due to algal respiration and cannot be interpreted as conventional BOD values.

RESULTS

The six ponds employed in this investigation were operated continuously for a period of fourteen weeks. During the first two weeks

operational difficulties prevented the collection of significant data. At the end of the second week the concentration of suspended solids in the six ponds ranged from 946 mg/l to 3042 mg/l. This time was taken as the starting point for the collection of data.

The results of the weekly analyses of the pond contents are presented in Table XVIII. The detebtion periods cited are averages of the nominal daily detention periods for each week. These values serve to indicate the relative magnitudes of the detention periods and show that the ponds were operated within the range of loadings associated with high rate stabilization ponds.

The values of the concentrations of suspended solids are given for the start and end of each week for each pond. From these values the total increase in the suspended solids for the week and the average between the initial and final suspended solids were calculated. The weekly increase in suspended solids concentration was exceedingly variable and probably depended more upon the particular species of algae which were predominant and the manipulation of the settled effluent than upon the actual algal production in the ponds. In a number of cases there was a decrease in the suspended solids in a pond over a period of one week.

When the effluent from a pond had been allowed to settle for one hour, there was generally a clear cut distinction between the settlable algal solids and the supernatent, although the supernatent had a definite green color. The values cited in the column headed Algal Vol.

TABLE XVIII

ALGAL ACCUMULATION AND BOD REDUCTION

Week	Pond	Deten. Period (days)	Suspended Solids Initial (mg/l)	Final (mg/l)	Inc. (mg/l)	Avg. (mg/l)	BOD Red. (%)	Algal Vol. (ml / 100 ml)	Vol. Index (ml / gm)
3	1	2.68	1422	2970	1548	2196	88.5	13	43.8
	2	3.18	3042	3958	916	3500	86.5	20	50.5
	3	2.99	1054	2686	1632	1870	90.8	12	44.7
	4	3.36	1372	3646	2274	2509	91.0	16	43.9
	5	6.00	946	2050	1114	1493	91.9	11	53.7
	6	3.81	1235	3058	1823	1823	95.9	13	42.5
4	1	3.05	2970	3506	536	3238	88.0	23	65.6
	2	5.68	3958	3524	-434	3741	88.6	13	36.9
	3	4.42	2686	2086	-600	2386	92.3	15	71.9
	4	2.54	3646	3616	-30	3431	85.8	36	99.9
	5	3.89	2050	2014	-36	2032	97.8	17	84.4
	6	6.32	3058	2230	-828	2644	97.0	12	53.8
5	1	3.08	3506	5597	2091	4552	73.7	22	39.3
	2	4.97	3524	3262	-262	3393	75.1	16	49.0
	3	4.19	2086	6532	4446	4309	94.5	21	32.2
	4	2.29	3616	7416	3800	5516	79.2	100	134.8
	5	4.33	2014	6086	4072	4050	92.2	22	36.1
	6	7.32	2230	4802	2572	3516	89.2	19	39.6
6	1	2.90	5597	6666	1069	6132	84.2	25	37.5
	2	5.28	3262	5066	1804	4164	91.0	22	43.4
	3	4.51	6532	6536	4	6534	84.4	35	53.5
	4	2.55	7416	5176	-2240	6296	76.6	100	193.2
	5	3.67	6086	5290	-796	5688	94.8	36	68.0
	6	7.23	4802	6688	1886	5745	64.4	20	29.9
7	1	2.70	6666	7372	706	7019	96.9	34	46.1
	2	4.05	5066	6680	1614	5873	96.6	20	29.9
	3	4.37	6536	7402	866	6969	91.9	36	48.6
	4	2.42	5176	6620	1444	5898	90.0	100	151.0
	5	4.00	5290	8392	3102	6841	95.9	34	40.5
	6	6.77	6688	6594	-94	6641	86.2	27	40.9
8	1	3.12	7372	9200	1828	8286	92.7	44	47.8
	2	5.83	6680	7050	370	6865	86.6	31	43.9
	3	4.12	7402	9302	1900	8352	92.4	35	37.6
	4	2.31	6620	9115	2496	7868	83.3	60	65.8
	5	4.70	8392	10648	2256	9520	93.6	44	41.3
	6	8.22	6594	7622	1028	7108	71.4	24	31.5

filtrate from the Gooch crucible filtration of the pond contents was visibly green but did not contain particles large enough to settle in 3 to 4 hours.

The rate of accumulation of algal solids seemed to be independent of the concentration of solids in the ponds. The maximum rates of accumulation as given in Table XVIII are probably indicative of what could be expected if all of the algae could be recovered and returned to the ponds. The greatest rate of accumulation found was 4446 mg/l per week in a pond with an average of 4309 mg/l of suspended solids. However, the pond having an average of 16,625 mg/l had an accumulation of 3950 mg/l in one week. Thus, solids concentrations as high as 1.6% seem to have no effect upon the rate of production of algae.

The detention period of a pond has a definite effect upon the BOD removal. Apparently, the BOD reduction is a maximum at a detention period of 4 to 5 days and will be less in ponds having either shorter or longer detention periods. This conclusion is consistent with the results obtained in high rate stabilization ponds in which the predominant species were unicellular green algae (1). The volume index of the settlable algal solids from the effluent definitely decreases with increasing detention period but is apparently independent of the total concentration of suspended solids present. Apparently, at the longer detention time the filamentous algae had more of a tendency to clump together. From this it could be concluded that detention periods in the range of 4 to 8 days would be optimum for the production of filamentous algae which may be harvested by sedimentation.

It might be thought that one method of eliminating the unicellular green algae from stabilization ponds with solids return would be to decrease the detention period to less than one day after an appreciable quantity of setteable solids had accumulated. However, from the data presented it would appear that decreasing the detention period would have an adverse effect upon the settlability of the filamentous algae and upon the BOD reduction.

The values for the volume index for the settleable algae are comparable with those usually obtained for activated sludge. It appears that it would be feasible to operate a stabilization pond with solids return with an algal concentration of around 1 gm/l in the pond and obtain a concentration of about 2.0-2.5 gm/l in the return algal solids. BOD removals of 85-90% could be expected. Operations on this basis should make harvesting of the excess algal solids economically more attractive.

CONCLUSIONS

1. It is possible to operate a stabilization pond so that high concentrations of filamentous algae are accumulated by settling the pond effluent and returning the settlable solids.

2. Stabilization ponds may be operated with algal concentrations in the range of 1-2% without causing a decrease in the BOD removal efficiency.

3. The rate of accumulation of algal solids in a stabilization pond with solids return is independent of the concentration of algal solids in the range 1 - 16 gm/l.

4. BOD removal in a stabilization pond with solids return is independent of algal solids concentration in the range 1 - 16 gm/l.

5. Best BOD removal in a stabilization pond with solids return is obtained with a detention period of 4-5 days.

6. The volume index of the settleable algal solids is greatest for ponds with short detention periods and decreases with increasing detention period in the range 2-8 days.

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fed from each reservoir was determined, the ponds were mixed by hand, and enough distilled water was added to each pond so that the effluent volume was equal to the influent volume for each pond.

Twelve series of experiments were run during the course of this investigation, each series consisting of four ponds operated in parallel. In the first ten series of experiments the ponds 1 through 4 were operated at detention periods of approximately 5, 10, 20, and 30 days respectively. During each series daily measurements of pH, alkalinity, and the packed cell volume of the algal cells in the pond were made at about 10 AM. It was found that when the ponds had been operating under uniform conditions for 30 to 40 days they reached an equilibrium condition and there was very little change in the determinations from day to day. At this point hourly measurements of pH and alkalinity were made on the ponds over a period of twenty four hours. The BOD of the effluent from each pond was determined and the series of experiments was terminated.

The BOD determinations were made on the pond effluents after they had been centrifuged to remove the algae. When the BOD determinations were made on the whole effluent with the algae present the results were extremely erratic. At times these determination would indicate a negative BOD probably due to algal photosynthesis during the incubation period and at other times the indicated BOD of the effluent was greater than the influent BOD probably due to respiratory utilization of oxygen by the algae. However, it was found that consistant results could be obtained when the BOD determinations were run on the centrifuged effluent.

In the first six series of experiments medium A and an incident light intensity of about 600 foot candles was employed. Since the results of these experiments did not turn out as expected the feed medium was changed to medium B and the incident light intensity was increased to 1000 foot candles for series VII through X. For series XI the light intensity was increased to 1600 foot candles; the detention period in each pond was approximately three days but the pH of the influent medium was varied from pond to pond. Series XII was the same as series XI except that the detention period in each pond was about twenty days.

RESULTS

The results of the twelve series of experiments is tabulated in Table XVI. The material in this table is self explanitary except for column three. The first letter in this column indicates whether the medium employed was medium A or B. The second letter of the column indicates whether the medium was fed to the pond continuously (C) for twenty hours each day or for the daylight (D) hours only. In series II, IV, and VI the pond were fed during the daylight hours only but the rate of feed was twice that which if would have been if the pond had been fed continuously at the same detention period.

The data concerning the maximum daytime pH attained are not entirely consistant. In general the maximum pH attained increased with increasing detention period but there are some exceptions to this generality especially in the series in which the influent medium

TABLE XVI

RESULTS OF POND STUDIES

Series No	Pond No	Medium Feed	Infl. pH	Infl. Alk. (mg/l)	Light Inten. (ft.-c.)	Deten. Period (days)	DOD Red. (%)	Max. pH	Pond Alk. (mg/l)
I	1	AC	8.0	183	600	5.6	68.8	9.5	122
I	2	AC	8.0	183	600	10.6	72.8	9.6	138
I	3	AC	8.0	183	600	22.5	78.5	9.7	152
I	4	AC	8.0	183	600	34.0	80.1	9.8	172
II	1	AD	8.0	183	600	5.4	72.3	9.3	130
II	2	AD	8.0	183	600	10.1	76.5	9.3	142
II	3	AD	8.0	183	600	19.0	75.7	9.4	158
II	4	AD	8.0	183	600	36.0	75.4	9.8	176
III	1	AC	7.2	148	600	5.5	92.0	9.2	126
III	2	AC	7.2	148	600	10.3	85.4	8.5	128
III	3	AC	7.2	148	600	18.0	81.7	9.6	133
III	4	AC	7.2	148	600	31.0	79.2	9.6	130
IV	1	AD	7.2	148	600	5.6	91.0	9.3	135
IV	2	AD	7.2	148	600	10.5	86.3	7.6	160
IV	3	AD	7.2	148	600	21.3	80.2	9.5	170
IV	4	AD	7.2	148	600	33.8	79.5	9.5	180
V	1	AC	6.0	112	600	5.5	93.2	9.3	132
V	2	AC	6.0	112	600	10.6	87.6	8.6	144
V	3	AC	6.0	112	600	22.4	80.4	9.6	156
V	4	AC	6.0	112	600	33.4	80.3	9.6	162
VI	1	AD	6.0	112	600	5.4	89.6	9.1	126
VI	2	AD	6.0	112	600	10.6	88.5	8.1	138
VI	3	AD	6.0	112	600	22.3	84.3	9.9	152
VI	4	AD	6.0	112	600	35.7	79.2	9.9	164
VII	1	BC	6.85	140	1000	4.9	89.0	8.1	148
VII	2	BC	6.85	140	1000	10.1	86.4	9.6	153
VII	3	BC	6.85	140	1000	19.2	82.8	10.2	165
VII	4	BC	6.85	140	1000	28.1	74.0	9.9	172
VIII	1	BC	8.8	204	1000	5.1	64.7	11.1	118
VIII	2	BC	8.8	204	1000	9.6	68.2	11.1	126
VIII	3	BC	8.8	204	1000	19.2	77.8	10.9	134
VIII	4	BC	8.8	204	1000	30.3	82.6	10.8	153
IX	1	BC	7.5	159	1000	4.7	95.6	11.2	148
IX	2	BC	7.5	159	1000	9.7	87.7	11.2	144
IX	3	BC	7.5	159	1000	19.2	83.6	11.1	136
IX	4	BC	7.5	159	1000	32.4	83.1	10.3	133
X	1	BC	7.5	159	1000	4.8	95.3	11.0	152
X	2	BC	7.5	159	1000	9.1	90.9	11.0	141
X	3	BC	7.5	159	1000	18.5	82.0	10.6	139
X	4	BC	7.5	159	1000	27.7	80.3	9.9	140
XI	1	BC	8.7	200	1600	3.3	74.8	11.5	137
XI	2	BC	7.5	159	1600	3.1	85.2	11.0	149
XI	3	BC	7.0	144	1600	3.2	89.4	10.8	151
XI	4	BC	6.5	130	1600	3.3	94.3	10.5	147

TABLE XVI
(Continued)

Series No	Pond No	Medium Feed	Infl. pH	Infl. Alk. (mg/l)	Light Inten. (ft.-c.)	Deten. Period (days)	BOD Red. (%)	Max. pH	Pond Alk. (mg/l)
XII	1	BC	8.5	192	1600	20.6	76.3	11.0	168
XII	2	BC	7.5	159	1600	19.2	78.0	10.9	152
XII	3	BC	7.0	144	1600	21.3	81.2	10.8	136
XII	4	BC	6.5	130	1600	19.7	85.1	10.6	138

had a high pH. Feeding the ponds only during the time the light was on apparently did decrease the maximum pH attained but this effect was not great. Decreasing the pH of the influent medium definitely did decrease the maximum daytime pH of the ponds with the shorter detention periods but did not have much effect on the ponds with detention periods of twenty days or greater. The maximum pH values attained were much greater when the incident light intensity was 1000 ft-candles than when it was 600 ft-candles.

The results of the 24-hour studies on the pH variation in the ponds presented a similar pattern in all experiments. Selected examples of this data are presented in figure 15. The only generalization which could be made after an extensive study of this data for all experiments was that the diurnal variation in pH was greater in the ponds having the shorter detention periods.

The data giving BOD reduction as a function of detention period for series I through VI are presented in figure 16. The data did not indicate any significant difference in BOD reduction in the ponds which were fed only during the day as opposed to those fed continuously. When

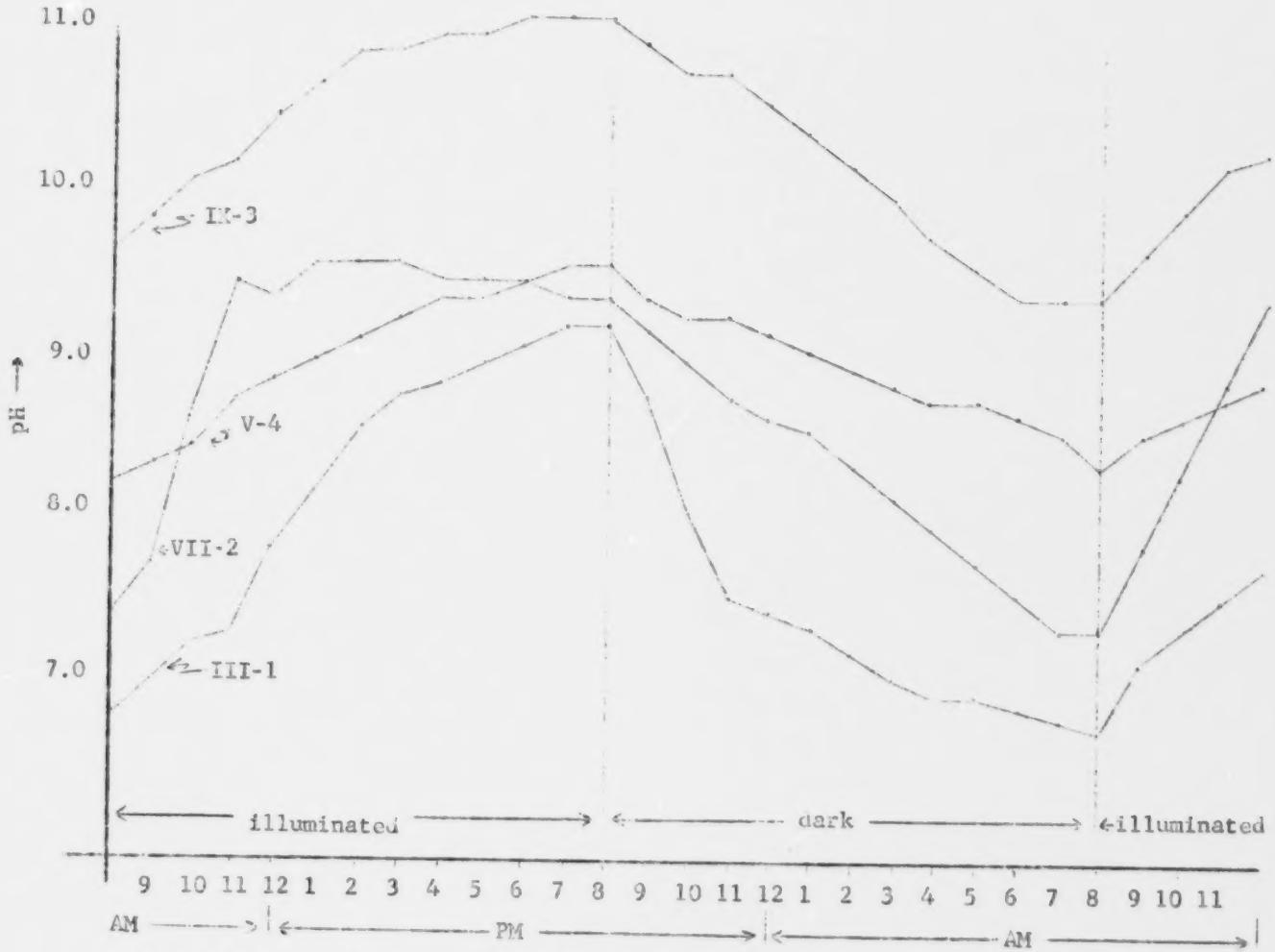

Fig. 15. Pattern of diurnal pH variation.

the influent medium had a pH of 8.0 an increase in BOD reduction with increasing detention period was observed. However, when the pH of the influent was 6.0 or 7.2 a decrease in the BOD reduction with increasing detention period was apparent. No different in the pattern was observed for an influent pH of 6.0 or 7.2. When the detention period in the pond was greater than twenty days the percentage BOD removal was always in the range 75-85%.

The results of BOD reduction versus detention period for series VII through X are presented in figure 17. The general pattern is the same as that observed in series I through VI but the effect of increasing detention period is more pronounced probably because with a higher incident light intensity the pH effect was greater. Again when the detention period of the pond was greater than twenty days the BOD removal was always in the range of 75-85%.

The results of the BOD removals attained in series XI and XII are plotted in figure 18. When the detention period in the pond was about three days there was a definite decrease in BOD removal with an increase in the influent pH. This same effect was noted in the ponds having a detention period of about twenty days; however, it was not as pronounced and the BOD removal was always in the 75-85% range.

As noted previously, it has been observed that the alkalinity of the effluent from stabilization ponds is usually less than that of the influent waste. In this series of experiments the alkalinity of the effluent was usually less than that of the influent medium. However, when the influent medium had a low pH and a low alkalinity the effluent

TABLE XVIII (cont.)

Week	Pond	Deten. Period (days)	Suspended Solids Initial (mg/l)	Final (mg/l)	Inc. (mg/l)	Avg. (mg/l)	BOD Red. (%)	Algal Vol. ($\frac{ml}{100\ ml}$)	Vol. Index ($\frac{ml}{gm}$)
9	1	2.60	9200	8554	-646	8877	76.8	38	44.4
	2	5.29	7050	7320	270	7185	89.9	23	31.4
	3	4.44	9302	8886	-416	9094	84.2	30	33.8
	4	2.31	9116	10176	1058	9645	69.2	100	98.3
	5	4.86	10648	9784	-864	10216	94.7	36	36.8
	6	4.04	7622	7912	290	7767	88.7	24	30.3
10	1	2.24	8554	10286	1732	9420	89.6	100	97.2
	2	4.04	7320	9514	2194	8417	96.1	27	28.4
	3	5.52	8886	8068	-818	8477	89.8	48	59.5
	4	3.19	10174	9702	-472	9938	90.0	100	103.1
	5	4.93	9784	9380	-404	9582	94.6	35	37.3
	6	6.95	7912	6512	-1400	7212	87.8	28	43.0
11	1	3.06	10286	10168	-118	10454	73.1	48	47.2
	2	4.54	9514	8706	-808	9220	88.2	32	36.8
	3	3.92	8068	9866	1798	8967	95.2	40	40.5
	4	2.56	9702	12064	2362	10883	82.9	52	43.1
	5	3.96	9380	11358	1978	10369	86.1	40	35.2
	6	3.85	6518	6358	-160	6438	79.8	24	37.7
12	1	6.14	10168	11322	1154	10745	88.4	45	39.7
	2	4.57	8706	9102	396	8904	92.8	39	42.8
	3	4.33	9866	10250	384	10058	93.6	42	41.0
	4	2.29	12064	14396	2332	13230	81.1	100	69.5
	5	3.34	11358	12440	1082	11899	78.0	48	38.6
	6	3.91	6358	8796	2432	7577	81.4	28	31.8
13	1	7.38	11322	10864	-458	11093	91.4	44	40.5
	2	3.54	9102	10200	1098	9651	98.2	44	43.1
	3	4.93	10250	11762	1512	11006	87.5	44	37.4
	4	2.64	14396	14650	254	14523	79.2	100	68.2
	5	4.30	12440	13942	1502	13191	88.2	52	37.3
	6	3.52	8796	7240	-1556	8018	71.3	30	41.4
14	1	2.91	10864	11870	1006	11367	90.5	44	37.0
	2	4.97	10200	11628	1428	10914	88.2	100	86.0
	3	5.50	11762	12922	1160	12344	88.0	44	34.1
	4	2.69	14650	18600	3950	16625	86.7	100	53.8
	5	3.95	13942	17128	3186	15535	82.8	68	39.7
	6	3.45	7240	8432	1172	7836	84.4	34	40.3

are the volume of the settlable algal solids divided by the volume of effluent and multiplied by one hundred in order to give a percentage value.

A plot of the volume occupied by the settlable solids after a half hour sedimentation versus the concentration of suspended solids is presented in Figure 19. Most of the points lie very close to a line whose slope indicates an average of 42.3 ml per gram of suspended solids. The average volume occupied by one gram of algal solids after thirty minutes' settling was termed the volume index by analogy to the sludge volume index for activated sludge. These values were calculated for each test and are given in Table XVIII. The average volume index for all tests was 52.5. The difference between this value and the slope of the line in Figure 19 is due to the fact that the algal volumes of 100% were ignored when the line was fitted to the rest of the points. The high values for the algal volume and the volume index occurred mostly in one pond (No. 4) and were associated with short detention periods and low BOD reductions.

The individual values for BOD reduction in each test were rather variable and showed considerable scatter when plotted versus either suspended solids concentration or detention period. In order to make the data somewhat more manageable, they were grouped according to the average suspended solids concentration and the average detention period. For each of these groups the average BOD reduction and the average increase in suspended solids were calculated. These data are presented in Table XIX and XX.

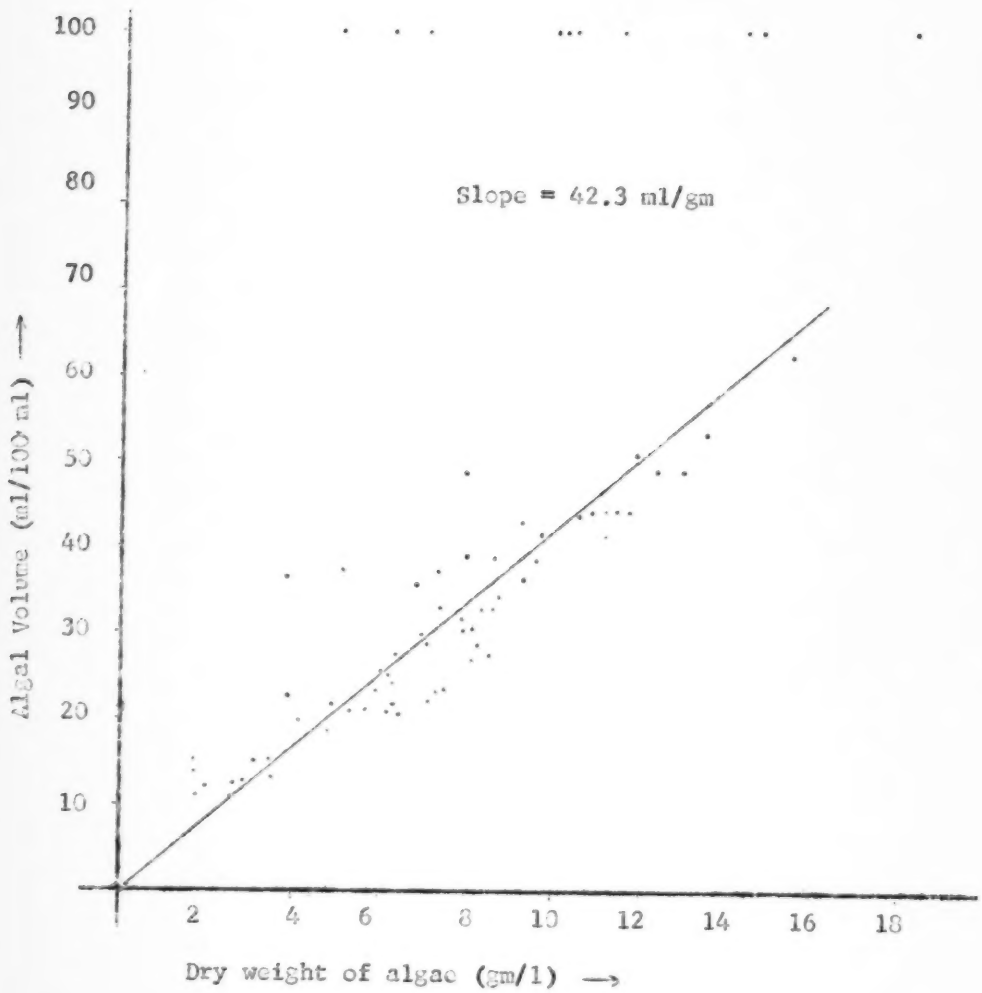

Fig. 19 Volume of settlable algal solids as a function of dry weight.

From the grouped data it is evident that the weekly increase in suspended solids concentration does not correlate with either average suspended solids concentration or the detention period. However, BOD reduction does seem to show some correlation with these parameters. The average BOD reduction is plotted against average suspended solids in Figure 20 and against detention period in Figure 21. Apparently, BOD reduction does decrease somewhat with increasing solids, but the actual amount of decrease is rather small. There was definitely better BOD removal in ponds having a detention period of 4 to 5 days than in ponds having either shorter or longer detention periods.

The data of Table XX indicate that there was a definite decrease in the volume index with an increase in detention period. However, there was apparently no direct relation between the volume index and the average BOD removal.

TABLE XIX

GROUPED DATA: SUSPENDED SOLIDS AND BOD REDUCTION

Avg. S.S. conc. (gm/l)	No. of Tests	Avg. S.S. Inc. (mg/l)	Max S.S. Inc. (mg/l)	Min S.S. Inc. (mg/l)	Avg. BOD Red. (%)	Max BOD Red. (%)	Min BOD Red. (%)
1-2	3	1523	1823	1114	92.6	95.9	90.8
2-3	5	586	2274	-600	93.3	97.8	88.5
3-4	6	549	2572	-434	85.5	89.2	75.1
4-5	4	3103	4446	1804	87.8	94.5	73.7
5-6	5	1509	3800	-796	85.0	96.6	64.4
6-7	8	405	3102	-2240	85.7	95.9	76.6
7-8	8	875	2496	-1400	85.5	96.9	71.4
8-9	8	637	2194	-1556	88.4	96.1	71.3
9-10	8	506	2256	-808	88.4	98.2	69.2
10-11	7	903	2362	-864	86.7	94.7	73.1
11-12	4	786	1512	-458	86.8	[illegible]	78.0
12-13	1	1160	1160	1160	88.0	88.0	88.0
13-14	2	1917	2332	1502	84.6	88.2	81.1
14-15	1	254	254	254	79.2	79.2	79.2
15-16	1	3186	3186	3186	82.6	82.6	82.6
16-17	1	3950	3950	3950	86.7	86.7	86.7

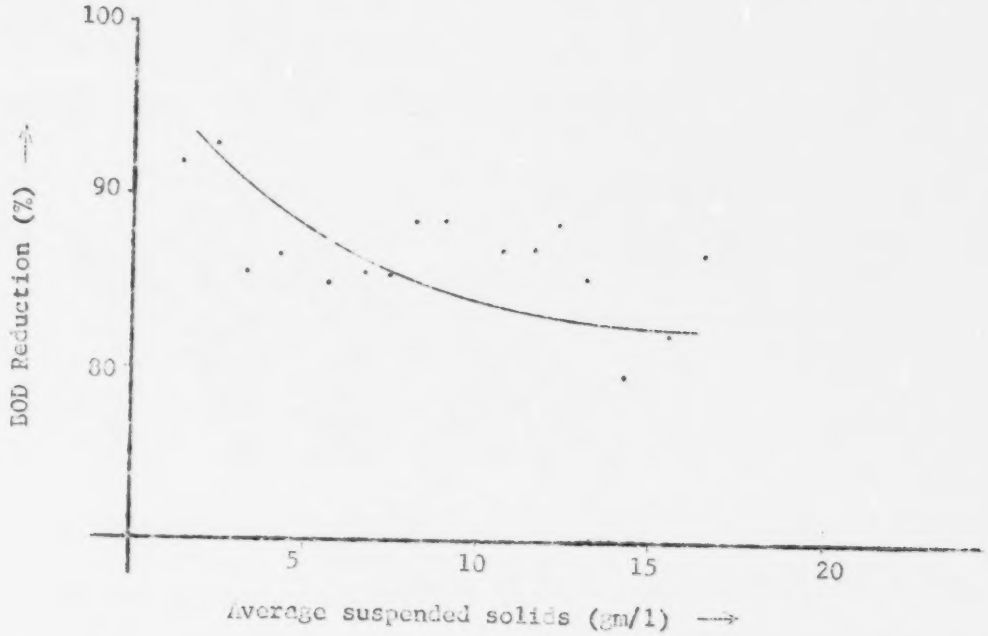

Fig. 20 BOD reduction as a function of suspended solids concentration.

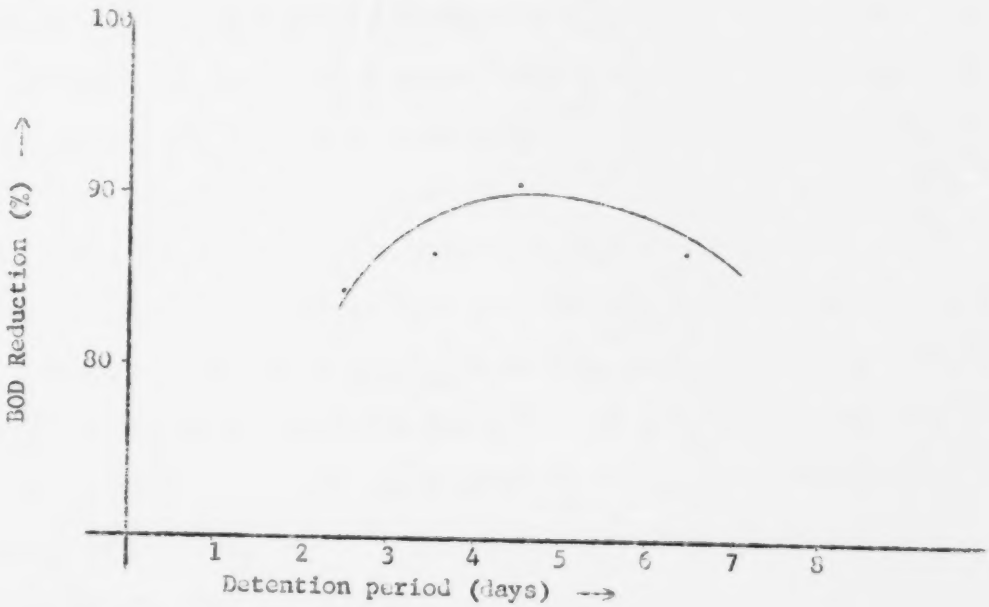

Fig. 21 BOD reduction as a function of detention period.

TABLE XX

GROUPED DATA: DETENTION PERIOD AND BOD REDUCTION

Detention Period (days)	No. of Tests	Increase in S.S. Avg. (mg/l)	Max. (mg/l)	Min. (mg/l)	BOD Reduction Avg. (%)	Max. (%)	Min. (%)	Avg. Vol Index (ml/gm)
2-3	17	1322	3950	-2240	84.2	90.8	69.2	78.1
3-4	19	1007	3186	-1556	86.6	90.2	71.3	49.5
4-5	21	1077	4446	-864	90.7	96.6	75.1	42.3
5-8	15	552	2572	-1400	86.7	97.0	71.4	41.5

DISCUSSION

For the interpretation of the data it was assumed that the suspended solids concentration was a measure of the concentration of algal solids which could be removed easily by filtration. The basis for this assumption was that microscopic examination showed that most of the suspended material consisted of algae. Some protozoa and worms were observed during the microscopic examination; however, in all cases they made up less than 10% of the mass of material observed. No agglomerations of bacteria and fungi were observed in any of the ponds.

Even though the settlable algae were returned to the ponds daily, the unicellular green algae, Chlorella and Scenedesmus, were able to maintain themselves in reasonable concentrations in all the ponds. No attempt was made to determine the relative densities of the filamentous and unicellular forms, but the general impression from the microscopic examinations was that the concentration of unicellular algae did not decrease as the total algal concentration increased. Invariably the

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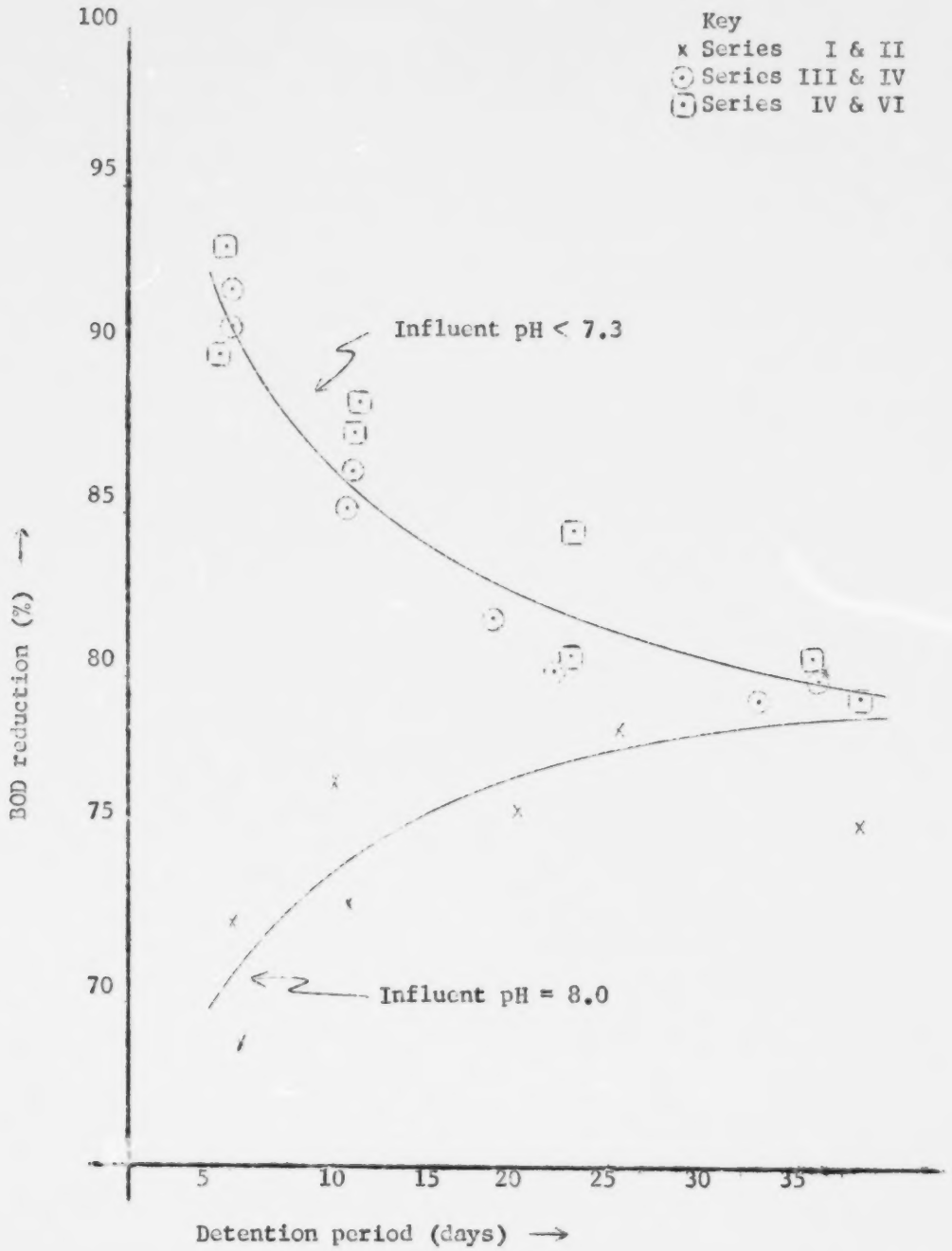

Fig. 16 BOD reduction as a function of detention period and influent pH (light intensity = 600 foot candles).

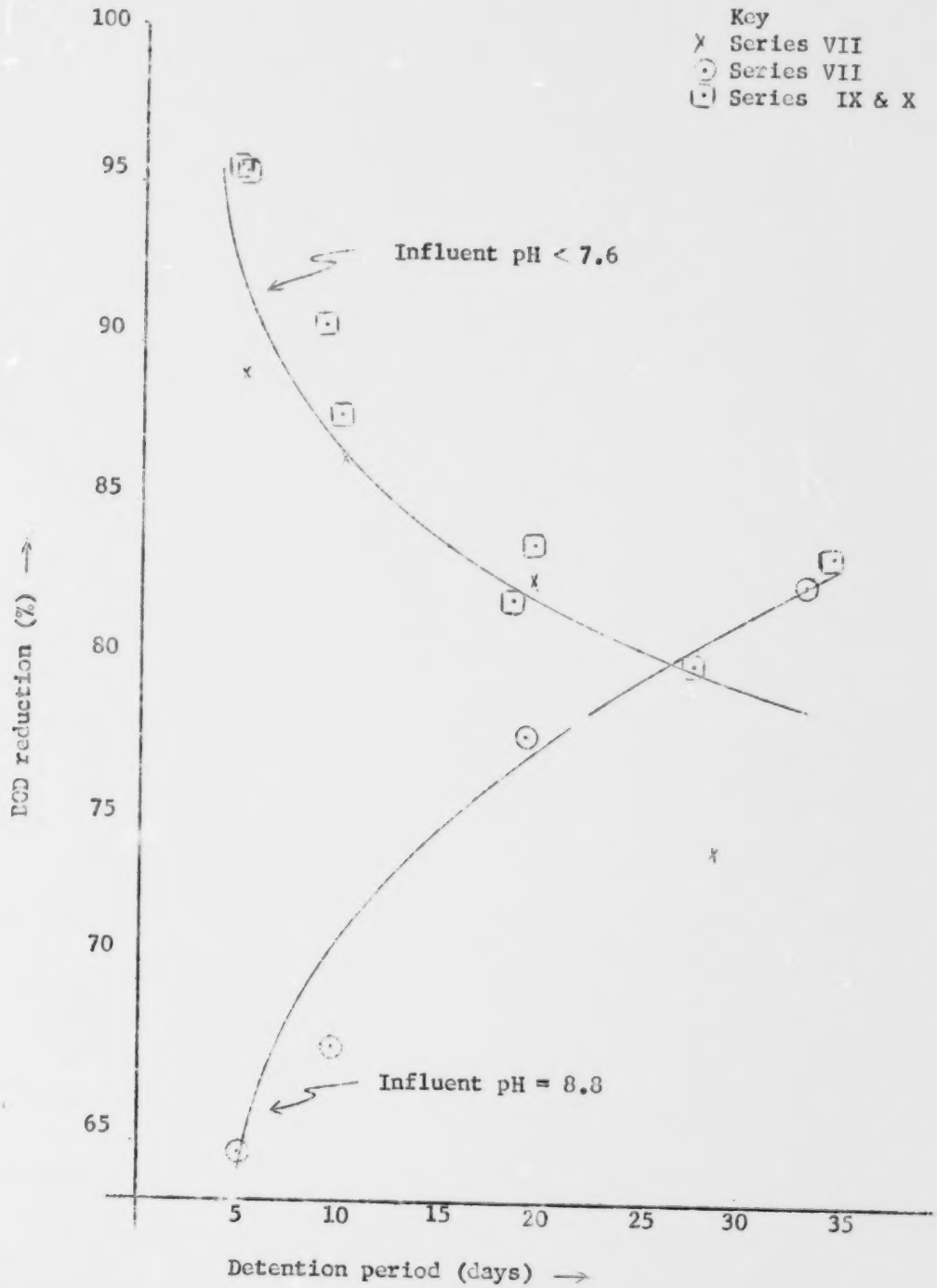

Fig. 17 BOD reduction as a function of detention period and influent pH (light intensity = 1000 foot candles).

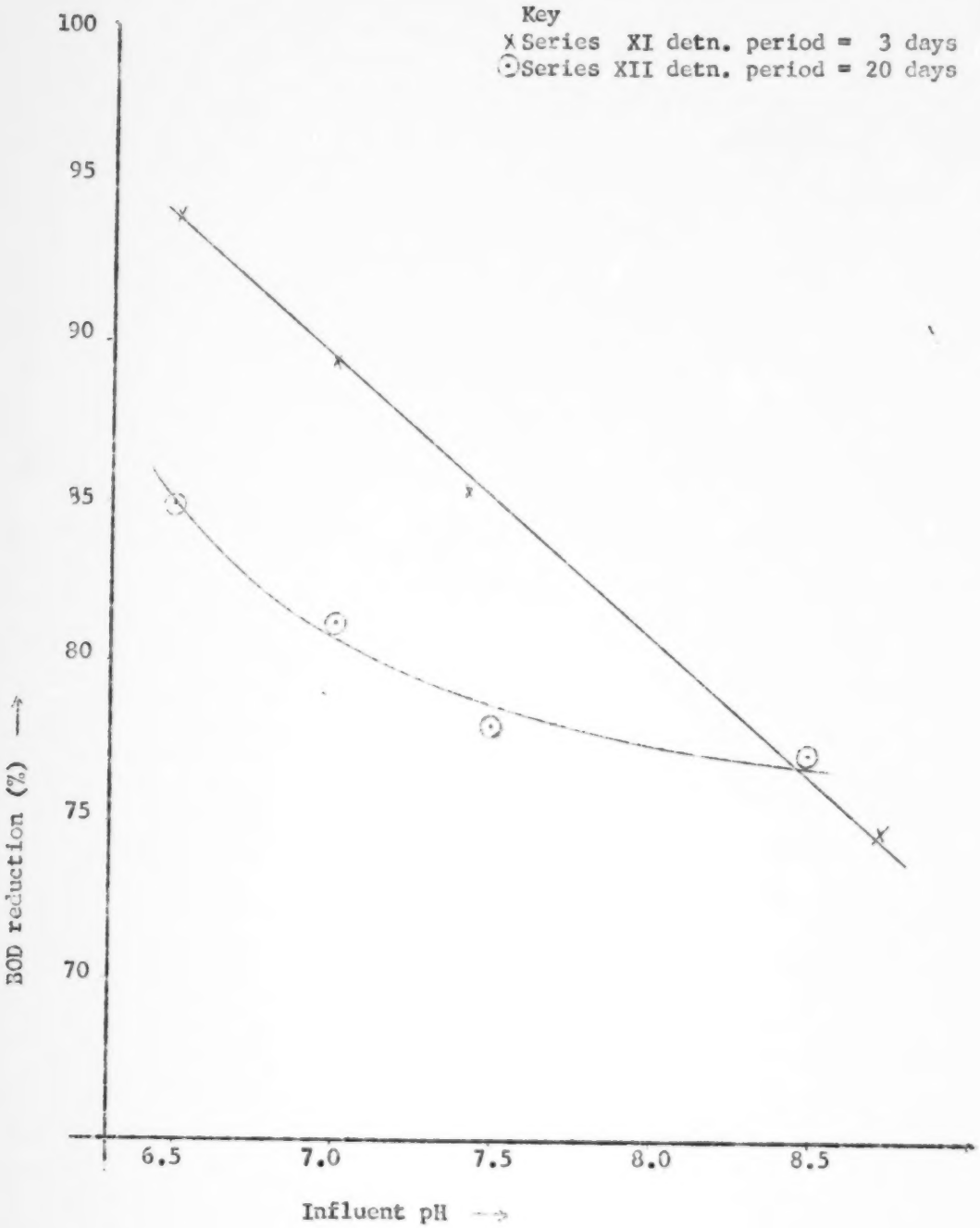

Fig. 18 BOD reduction as a function of influent pH.

had a higher alkalinity than the influent. From this observation it is evident that mechanisms other than the nitrogen metabolism of the algae are involved in the alkalinity changes which occur in stabiliaztion ponds.

DISCUSSION

The interpretation of the results of this investigation was hampered by the fact that no reasonable perameter to describe the pH variation in the pond and the effect of pH variation was developed. Oswald (63) suggested a perameter, named pH duration, which was defined as:

pH duration = (Max. pH-8.0) (Periodicity) (Detention Period).

When BOD reduction was plotted as a function of pH duration for the data collected in this investigation no overall correlation could be found. For the individual series in which the influent pH was 7.2 or less a correlation was observed but the detention period had a much greater effect on the value of pH duration than did the maximum daytime pH.

Other perameters which were tried as expressions of the pH effect were percentage of time the pH was above 9.0, ratio of maximum pH to influent pH, and the maximum daytime pH. No correlation was obtained between any of the perameters and the BOD reduction.

The fact that no correlation between any of these perameters and the BOD reduction could be taken to indicate that the pH variation had no effect upon the stabilization of the organic matter in the ponds. However, when the effect of pH variation is isolated from the effect of

variation of the detention period, which is equivalent to variation of loading, there is a distinct decrease in the BOD reduction with an increase in the influent pH as shown by the data from series XI and XII. Apparently pH does have an effect upon the reduction of BOD in a stabilization pond but so do a number of other factors.

The effect of detention period upon BOD removal seems to be greatly influenced by the influent pH. When the influent pH is greater than 7.2 increasing detention period increased BOD removal but when the influent pH is 7.2 or less the converse phenomen is observed. This reversion of effect seems to be more pronounced when the incident light intensity is greater. Since the light intensities employed in this investigation were considerably below those of bright sunlight it is possible that this effect might have a greater importance in real stabilization ponds than was observed in the laboratory scale ponds.

In all the ponds which were operated at detention periods of 20 days or greater the BOD removals were in the range of 75-85%. Since conventional stabilization ponds are operated at detention periods greater than 20 days it is likely that pH effects are not so great in these ponds. However, since the influent pH of sewage to conventional ponds is usually less than 7.5 it is also likely that better BOD removals could be achieved in ponds with shorter detention periods.

The high rate stabilization ponds which depend almost entirely upon photosynthetic oxygenation to prevent anaerobic conditions are operated at much shorter detention periods than conventional ponds (1).

For ponds of this type the pH of the influent waste will have a pronounced effect upon BOD removal and apparently it is quite important to assure that the influent pH for these ponds is not above 7.2.

CONCLUSIONS

1. Loading a stabilization pond only during the hours of daylight does not appear to be a practical method of preventing high pH values in the pond.

2. Lowering the pH of the influent waste does not prevent a rapid increase in pond pH during the daytime but it does prevent the pH from reaching extremely high values.

3. Decreasing the pH of the influent waste does increase the percentage BOD removal in a stabilization pond.

4. Control of influent pH does not have a pronounced effect upon BOD removal in the conventional type of stabilization ponds but it does seem to be an important factor in achieving high BOD removals in high rate type stabilization ponds.